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(54) Title: NOVEL <i>BACILLUS THURINGIENSIS</i> ISOLATES FOR CONTROLLING ACARIDES			
(57) Abstract Disclosed and claimed are <i>Bacillus thuringiensis</i> isolates designated <i>B.t.</i> PS50C, <i>B.t.</i> PS86A1, <i>B.t.</i> PS69D1, <i>B.t.</i> PS72L1, <i>B.t.</i> PS75J1, <i>B.t.</i> PS83E5, <i>B.t.</i> PS45B1, <i>B.t.</i> PS24J, <i>B.t.</i> PS94R3, <i>B.t.</i> PS17, <i>B.t.</i> PS62B1 and <i>B.t.</i> PS74G1 which are active against acaride pests. Thus, these isolates, or mutants thereof, can be used to control such pests. Further, genes encoding novel δ -endotoxins can be removed from these isolates and transferred to other host microbes, or plants. Expression of the δ -endotoxins in microbe hosts results in the control of acaride pests, whereas transformed plants become resistant to acaride pests.			

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DESCRIPTIONNOVEL *BACILLUS THURINGIENSIS* ISOLATES
FOR CONTROLLING ACARIDESCross-Reference to a Related Application

This is a continuation-in-part of co-pending application Serial No. 07/693,210, filed on April 30, 1991. This is also a continuation-in-part of application Serial No. 07/768,141, filed on September 30, 1991 which is a continuation-in-part of application Serial No. 07/759,248, filed on September 13, 1991.

Background of the Invention

The spore-forming microorganism *Bacillus thuringiensis* (*B.t.*) produces the best-known insect toxin. The toxin is a protein, designated as δ -endotoxin. It is synthesized by the *B.t.* sporulating cell. The toxin, upon being ingested in its crystalline form by susceptible insect larvae, is transformed into biologically active moieties by the insect gut juice proteases. The primary target is insect cells of the gut epithelium, which are rapidly destroyed. Experience has shown that the activity of the *B.t.* toxin is so high that only nanogram amounts are required to kill susceptible insects.

The reported activity spectrum of *B.t.* covers insect species within the order Lepidoptera, which is a major insect problem in agriculture and forestry. The activity spectrum also includes the insect order Diptera, wherein reside mosquitoes and blackflies. See Couch, T.L., (1980) "Mosquito Pathogenicity of *Bacillus thuringiensis* var. *israelensis*," *Developments in Industrial Microbiology*, 22:61-67; Beegle, C.C., (1978) "Use of Entomogeneous Bacteria in Agroecosystems," *Developments in Industrial Microbiology*, 20:97-104.

U.S. Patent 4,771,131 discloses a toxin gene isolated from a strain of *Bacillus thuringiensis*. This gene encodes a toxin which is active against beetles of the order Coleoptera.

There have been published reports concerning the use of *Bacillus thuringiensis* preparations for the control of mites. These publications are as follow:

Royalty, R.N., Hall, F.R. and Taylor, R.A.J. 1990. Effects of thuringiensin on *Tetranychus urticae* (Acari: Tetranychidae) mortality, fecundity, and feeding. *J. Econ. Entomol.* 83:792-798.

Neal, J.W., Lindquist, R.K., Gott, K.M. and Casey, M.L. 1987. Activity of the thermostable beta-exotoxin of *Bacillus thuringiensis* Berliner on *Tetranychus urticae* and *Tetranychus cinnabarinus*. *J. Agric. Entomol.* 4:33-40.

Viayen, P., Impe, G. and Van Semaille, R. 1978. Effect of a commercial preparation of Bacillus thuringiensis on the spider mite Tetranychus urticae Koch. (Acari: Tetranychidae). Mededelingen 43:471-479.

5 In the above published studies, the active ingredient in the B.t. preparations was beta-exotoxin (also called thuringiensin).

U.S. Patent No. 4,695,455 concerns methods and compositions for preparing and using biological pesticides, where the pesticides are encapsulated in non-proliferating cells.

U.S. Patent No. 4,849,217 concerns B.t. isolates active against the alfalfa weevil.

10 Brief Summary of the Invention

The subject invention concerns Bacillus thuringiensis isolates and toxins which have acaricidal properties. Unlike published reports of the use of B.t. δ -exotoxins to control mites, the subject invention isolates express δ -endotoxins which control mites. The use of δ -endotoxins is highly advantageous in view of the known general toxicity of δ -exotoxins to humans and animals.

15 More specifically, the subject invention concerns Bacillus thuringiensis isolates designated B.t. PS50C, B.t. PS86A1, B.t. PS69D1, B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t. PS24J, B.t. PS94R3, B.t. PS17, B.t. PS62B1 and B.t. PS74G1.

20 The B.t. isolates of the subject invention are toxic to the Two Spotted Spider Mite, Tetranychus urticae. Thus, these isolates can be used to control this mite. Further, the δ -endotoxins from these B.t. isolates can be isolated by standard procedures, e.g. ion exchange, and formulated by standard procedures to control the Two Spotted Spider Mite. These B.t. isolates can also be used against non-phytophagous mites such as acarid pests of livestock, fowl and stored products. Still further, the gene(s) from the B.t. isolates of the invention which encode the acaricidal toxin can be cloned from the isolates and then used to transform other hosts, e.g., prokaryotic, eukaryotic or plants, which transformed host can be used to control mites, or, in the case of transgenic plants, be resistant to mites.

30 Brief Description of the Drawings

FIGURES 1, 2A and 2B are photographs of 12% SDS polyacrylamide gels showing alkali-soluble proteins of the isolates of the invention.

Brief Description of the Sequences

35 SEQ ID NO. 1 discloses the DNA of 17a.

SEQ ID NO. 2 discloses the amino acid sequence of the toxin encoded by 17a.

SEQ ID NO. 3 discloses the DNA of 17b.

SEQ ID NO. 4 discloses the amino acid sequence of the toxin encoded by 17b.

SEQ ID NO. 5 is the nucleotide sequence of gene 33F2.

SEQ ID NO. 6 is the nucleotide sequence of a gene from 52A1.

SEQ ID NO. 7 is the amino acid sequence of the protein expressed by the gene from 52A1.

SEQ ID NO. 8 is the nucleotide sequence of a gene from 69D1.

5 SEQ ID NO. 9 is the amino acid sequence of the protein expressed by the gene from 69D1.

SEQ ID NO. 10 is the DNA coding for the amino acid sequence of SEQ ID NO. 13.

10 SEQ ID NO. 11 is the amino acid sequence of a probe which can be used according to the subject invention.

SEQ ID NO. 12 is the N-terminal amino acid sequence of 17a.

SEQ ID NO. 13 is the N-terminal amino acid sequence of 17b.

SEQ ID NO. 14 is the N-terminal amino acid sequence of 52A1.

SEQ ID NO. 15 is the N-terminal amino acid sequence of 69D1.

15 SEQ ID NO. 16 is a synthetic oligonucleotide derived from 17.

SEQ ID NO. 17 is an oligonucleotide probe designed from the N-terminal amino acid sequence of 52A1.

SEQ ID NO. 18 is the synthetic oligonucleotide probe designated as 69D1-D.

SEQ ID NO. 19 is the forward oligonucleotide primer from 63B.

20 SEQ ID NO. 20 is the reverse complement primer to SEQ ID NO. 29, used according to the subject invention.

SEQ ID NO. 21 is the DNA coding for the primer of SEQ ID NO. 31.

SEQ ID NO. 22 is a forward primer according to the subject invention.

SEQ ID NO. 23 is a probe according to the subject invention.

25 SEQ ID NO. 24 is a probe according to the subject invention.

SEQ ID NO. 25 is a probe according to the subject invention.

SEQ ID NO. 26 is a forward primer according to the subject invention.

SEQ ID NO. 27 is the nucleotide sequence of a gene from PS50C.

30 SEQ ID NO. 28 is the amino acid sequence of the protein expressed by the gene from PS50C.

SEQ ID NO. 29 is the nucleotide sequence of a gene from PS86A1.

SEQ ID NO. 30 is the amino acid sequence of the protein expressed by the gene from PS86A1.

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Detailed Disclosure of the Invention

The subject invention concerns B.t. δ -endotoxins having acaricidal activity. In addition to having acaricidal activity, the toxins of the subject invention may have one or more of the following characteristics:

1. A high degree of amino acid homology with specific toxins disclosed herein.
2. A DNA sequence encoding the toxin which hybridizes with probes or genes disclosed herein.
- 5 3. A nucleotide sequence which can be amplified using primers disclosed herein.
4. Immunoreactivity to an antibody raised to a specific toxin disclosed herein.

10 Acaride-active toxins according to the subject invention are specifically exemplified herein by the toxins encoded by the genes designated 17a, 17b, and 69D1. Since these toxins are merely exemplary of the toxins presented herein, it should be readily apparent that the subject invention further comprises toxins from the other disclosed isolates as well as equivalent toxins (and nucleotide sequences coding for equivalent toxins) having the same or similar biological activity of the specific toxins disclosed or claimed herein. These equivalent
15 toxins will have amino acid homology with the toxins disclosed and claimed herein. This amino acid homology will typically be greater than 50%, preferably be greater than 75%, and most preferably be greater than 90%. The amino acid homology will be highest in certain critical regions of the toxin which account for biological activity or are involved in the determination of three-dimensional configuration which ultimately is responsible for the
20 biological activity. In this regard, certain amino acid substitutions are acceptable and can be expected if these substitutions are in regions which are not critical to activity or are conservative amino acid substitutions which do not affect the three-dimensional configuration of the molecule. For example, amino acids may be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one
25 class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution does not materially alter the biological activity of the compound. Table 1 provides a listing of examples of amino acids belonging to each class.

Table 1	
Class of Amino Acid	Examples of Amino Acids
Nonpolar	Ala, Val, Leu, Ile, Pro, Met, Phe, Trp
Uncharged Polar	Gly, Ser, Thr, Cys, Tyr, Asn, Gln
Acidic	Asp, Glu
Basic	Lys, Arg, His

35 In some instances, non-conservative substitutions can also be made. The critical factor is that these substitutions must not significantly detract from the biological activity of

the toxin. The information presented in the generic formulae of the subject invention provides clear guidance to the person skilled in this art in making various amino acid substitutions.

The B.t. isolates of the invention have the following characteristics:

	<u>Strain</u>		<u>Crystal Type</u>	<u>Approx. Mol. Wt. of Proteins (kDa)</u>
5	<u>B. thuringiensis</u>	PS50C	Sphere	135 doublet
	<u>B. thuringiensis</u>	PS86A1	Multiple	45, 58
	<u>B. thuringiensis</u>	PS69D1	Elongated	34, 48, 145
	<u>B. thuringiensis</u>	PS72L1	Long rectangle	42, 50
10	<u>B. thuringiensis</u>	PS75J1	Amorphic	63, 74, 78, 84
	<u>B. thuringiensis</u>	PS83E5	Multiple	37, 42
	<u>B. thuringiensis</u>	PS24J	Long	51, 48, 43
	<u>B. thuringiensis</u>	PS94R3	Long	50, 43, 42
	<u>B. thuringiensis</u>	PS45B1	Multiple	150, 135, 35
15	<u>B. thuringiensis</u>	PS17	Long	155, 145, 128
	<u>B. thuringiensis</u>	PS62B1	Attached multiple	35
	<u>B. thuringiensis</u>	PS74G1	Amorphic	148, 112, 104, 61

20 Additionally, the isolates have the following common characteristics:

Colony morphology — large colony, dull surface, typical B.t.

Vegetative cell morphology — typical B.t.

25 The toxins of the subject invention can be accurately characterized in terms of the shape and location of crystal toxin inclusions. Specifically, acaride-active inclusions typically remain attached to the spore after cell lysis. These inclusions are not inside the exosporium, as in previous descriptions of attached inclusions, but are held within the spore by another mechanism. Inclusions of the acaride-active isolates are typically amorphic, generally long and/or multiple. These inclusions are distinguishable from the larger round/amorphic inclusions that remain attached to the spore. No B.t. strains that fit this description have been found to have activity against the conventional targets—Lepidoptera, Diptera, or Colorado Potato Beetle. We have found a very high correlation between this crystal structure and acaride activity.

35 The genes and toxins according to the subject invention include not only the full length sequences disclosed herein but also fragments of these sequences, or fusion proteins, which retain the characteristic acaricidal activity of the sequences specifically exemplified herein.

40 It should be apparent to a person skilled in this art that genes coding for acaride-active toxins can be identified and obtained through several means. The specific genes may be obtained from a culture depository as described below. These genes, or portions thereof, may be constructed synthetically, for example, by use of a gene machine. Variations of these genes may be readily constructed using standard techniques for making point mutations. Also, fragments of these genes can be made using commercially available exonucleases or endonucleases according to standard procedures. For example, enzymes such as Bal31 or site-directed mutagenesis can be used to systematically cut off nucleotides from the ends of these

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genes. Also, genes which code for active fragments may be obtained using a variety of other restriction enzymes. Proteases may be used to directly obtain active fragments of these toxins.

Equivalent toxins and/or genes encoding these equivalent toxins can also be located from B.t. isolates and/or DNA libraries using the teachings provided herein. There are a number of methods for obtaining the acaride-active toxins of the instant invention which occur in nature. For example, antibodies to the acaride-active toxins disclosed and claimed herein can be used to identify and isolate other toxins from a mixture of proteins. Specifically, antibodies may be raised to the acaride-active toxins using procedures which are well known in the art. These antibodies can then be used to specifically identify equivalent toxins with the characteristic acaricidal activity by immunoprecipitation, enzyme linked immunoassay (ELISA), or Western blotting. Antibodies to the toxins disclosed herein, or to equivalent toxins, or fragments of these toxins, can readily be prepared using standard procedures in this art. The genes coding for these toxins can then be obtained from the microorganism.

A further method for identifying the toxins and genes of the subject invention is through the use of oligonucleotide probes. These probes are nucleotide sequences having a detectable label. As is well known in the art, if the probe molecule and nucleic acid sample hybridize by forming a strong bond between the two molecules, it can be reasonably assumed that the probe and sample are essentially identical. The probe's detectable label provides a means for determining in a known manner whether hybridization has occurred. Such a probe analysis provides a rapid method for identifying nematocidal endotoxin genes of the subject invention.

The nucleotide segments which are used as probes according to the invention can be synthesized by use of DNA synthesizers using standard procedures. In the use of the nucleotide segments as probes, the particular probe is labeled with any suitable label known to those skilled in the art, including radioactive and non-radioactive labels. Typical radioactive labels include ^{32}P , ^{125}I , ^{35}S , or the like. A probe labeled with a radioactive isotope can be constructed from a nucleotide sequence complementary to the DNA sample by a conventional nick translation reaction, using a DNase and DNA polymerase. The probe and sample can then be combined in a hybridization buffer solution and held at an appropriate temperature until annealing occurs. Thereafter, the membrane is washed free of extraneous materials, leaving the sample and bound probe molecules typically detected and quantified by autoradiography and/or liquid scintillation counting.

Non-radioactive labels include, for example, ligands such as biotin or thyroxine, as well as enzymes such as hydrolases or peroxidases, or the various chemiluminescers such as luciferin, or fluorescent compounds like fluorescein and its derivatives. The probe may also be labeled at both ends with different types of labels for ease of separation, as, for example, by using an isotopic label at the end mentioned above and a biotin label at the other end.

Duplex formation and stability depend on substantial complementarity between the two strands of a hybrid, and, as noted above, a certain degree of mismatch can be tolerated.

Therefore, the probes of the subject invention include mutations (both single and multiple), deletions, insertions of the described sequences, and combinations thereof, wherein said mutations, insertions and deletions permit formation of stable hybrids with the target polynucleotide of interest. Mutations, insertions, and deletions can be produced in a given polynucleotide sequence in many ways, and these methods are known to an ordinarily skilled artisan. Other methods may become known in the future.

The known methods include, but are not limited to:

- (1) synthesizing chemically or otherwise an artificial sequence which is a mutation, insertion or deletion of the known sequence;
- (2) using a probe of the present invention to obtain via hybridization a new sequence or a mutation, insertion or deletion of the probe sequence; and
- (3) mutating, inserting or deleting a test sequence in vitro or in vivo.

It is important to note that the mutational, insertional, and deletional variants generated from a given probe may be more or less efficient than the original probe. Notwithstanding such differences in efficiency, these variants are within the scope of the present invention.

Thus, mutational, insertional, and deletional variants of the disclosed test sequences can be readily prepared by methods which are well known to those skilled in the art. These variants can be used in the same manner as the instant probes so long as the variants have substantial sequence homology with the probes. As used herein, substantial sequence homology refers to homology which is sufficient to enable the variant to function in the same capacity as the original probe. Preferably, this homology is greater than 50%; more preferably, this homology is greater than 75%; and most preferably, this homology is greater than 90%. The degree of homology needed for the variant to function in its intended capacity will depend upon the intended use of the sequence. It is well within the skill of a person trained in this art to make mutational, insertional, and deletional mutations which are designed to improve the function of the sequence or otherwise provide a methodological advantage.

Specific nucleotide probes useful, according to the subject invention, in the rapid identification of acaricide-active genes can be prepared utilizing the sequence information provided herein.

The potential variations in the probes listed is due, in part, to the redundancy of the genetic code. Because of the redundancy of the genetic code, i.e., more than one coding nucleotide triplet (codon) can be used for most of the amino acids used to make proteins. Therefore different nucleotide sequences can code for a particular amino acid. Thus, the amino acid sequences of the *Bt* toxins and peptides can be prepared by equivalent nucleotide sequences encoding the same amino acid sequence of the protein or peptide. Accordingly, the subject invention includes such equivalent nucleotide sequences. Also, inverse or complement sequences are an aspect of the subject invention and can be readily used by a person skilled in this art. In addition it has been shown that proteins of identified structure and function

may be constructed by changing the amino acid sequence if such changes do not alter the protein secondary structure (Kaiser, E.T. and Kezdy, F.J. [1984] Science 223:249-255). Thus, the subject invention includes mutants of the amino acid sequence depicted herein which do not alter the protein secondary structure, or if the structure is altered, the biological activity is substantially retained. Further, the invention also includes mutants of organisms hosting all or part of a toxin encoding a gene of the invention. Such microbial mutants can be made by techniques well known to persons skilled in the art. For example, UV irradiation can be used to prepare mutants of host organisms. Likewise, such mutants may include asporogenous host cells which also can be prepared by procedures well known in the art.

The B.t. isolates of the invention, and mutants thereof, can be cultured using standard known media and fermentation techniques. Upon completion of the fermentation cycle, the bacteria can be harvested by first separating the B.t. spores and crystals from the fermentation broth by means well known in the art. The recovered B.t. spores and crystals can be formulated into a wettable powder, a liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers and other components to facilitate handling and application for particular target pests. The formulation and application procedures are all well known in the art and are used with commercial strains. The novel B.t. isolates, and mutants thereof, can be used to control target pests.

The cultures of the subject invention were deposited in the Agricultural Research Service Patent Culture Collection (NRRL), Northern Regional Research Center, 1815 North University Street, Peoria, Illinois, 61604 USA.

<u>Culture</u>	<u>Accession No.</u>	<u>Deposit Date</u>
<u>B.t.</u> PS50C	NRRL B-18746	January 9, 1991
<u>B.t.</u> PS86A1	NRRL B-18400	August 16, 1988
<u>B.t.</u> PS69D1	NRRL B-18247	July 28, 1987
<u>B.t.</u> PS72L1	NRRL B-18780	March 7, 1991
<u>B.t.</u> PS75J1	NRRL B-18781	March 7, 1991
<u>B.t.</u> PS83E5	NRRL B-18782	March 7, 1991
<u>B.t.</u> PS45B1	NRRL B-18396	August 16, 1988
<u>B.t.</u> PS24J	NRRL B-18881	August 30, 1991
<u>B.t.</u> PS94R3	NRRL B-18882	August 30, 1991
<u>B.t.</u> PS17	NRRL B-18243	July 28, 1987
<u>B.t.</u> PS62B1	NRRL B-18398	August 16, 1988
<u>B.t.</u> PS74G1	NRRL B-18397	August 16, 1988
<i>E. coli</i> NM522(pMYC 2321)	NRRL B-18770	February 14, 1991
<i>E. coli</i> NM522(pMYC 2317)	NRRL B-18816	April 24, 1991
<i>E. coli</i> NM522(pMYC 1627)	NRRL B-18651	May 11, 1990
<i>E. coli</i> NM522(pMYC 1628)	NRRL B-18652	May 11, 1990
<i>E. coli</i> NM522(pMYC 1638)	NRRL B-18751	January 11, 1991

E. coli NM522(pMYC 1638)

NRRL B-18769

February 14, 1991

The subject cultures have been deposited under conditions that assure that access to the cultures will be available during the pendency of this patent application to one determined by the Commissioner of Patents and Trademarks to be entitled thereto under 37 CFR 1.14 and 35 U.S.C. 122. These deposits are available as required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny, are filed. However, it should be understood that the availability of a deposit does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

Further, the subject culture deposits will be stored and made available to the public in accord with the provisions of the Budapest Treaty for the Deposit of Microorganisms, i.e., they will be stored with all the care necessary to keep them viable and uncontaminated for a period of at least five years after the most recent request for the furnishing of a sample of a deposit, and in any case, for a period of at least thirty (30) years after the date of deposit or for the enforceable life of any patent which may issue disclosing a culture. The depositor acknowledges the duty to replace a deposit should the depository be unable to furnish a sample when requested, due to the condition of a deposit. All restrictions on the availability to the public of the subject culture deposits will be irrevocably removed upon the granting of a patent disclosing them.

Upon applying an acaricidal-effective amount of a microbe, or toxin, as disclosed herein, in a suitable acaricidal formulation to the environment of the target pest, there is obtained effective control of these pests. An acaricidal-effective amount can vary from about 1 to about 12 l/ha, depending upon the nature and quantity of the pests to be controlled, the time of year, temperature, humidity, and other known factors which may affect a bioinsecticide. It is well within the skill of those trained in this art to determine the quantity of bioinsecticide to apply in order to obtain effective control of target pests.

The intracellular δ -endotoxin protein can be combined with other insecticidal proteins (including those obtained from sources other than *Bacillus thuringiensis*) to increase the spectrum of activity to give complete control of target pests.

The *B.t.* cells may be formulated in a variety of ways. They may be employed as wettable powders, granules or dusts, by mixing with various inert materials, such as inorganic minerals (phyllosilicates, carbonates, sulfates, phosphates, and the like) or botanical materials (powdered corncobs, rice hulls, walnut shells, and the like). The formulations may include spreader-sticker adjuvants, stabilizing agents, other pesticidal additives, or surfactants. Liquid formulations may be aqueous-based or non-aqueous and employed as foams, gels, suspensions, emulsifiable concentrates, or the like. The ingredients may include rheological agents, surfactants, emulsifiers, dispersants, and polymers.

The pesticidal concentration will vary widely depending upon the nature of the particular formulation, particularly whether it is a concentrate or to be used directly. The

pesticide will be present in at least 1% by weight and may be 100% by weight. The dry formulations will have from about 1-95% by weight of the pesticide while the liquid formulations will generally be from about 1-60% by weight of the solids in the liquid phase. The formulations will generally have from about 10^2 to about 10^4 cells/mg. These formulations will be administered at about 50 mg (liquid or dry) to 1 kg or more per hectare.

The formulations can be applied to the environment of the target pest(s), e.g., plants, livestock, fowl, soil or water, by spraying, dusting, sprinkling, or the like.

The toxin genes harbored by the novel isolates of the subject invention can be introduced into a wide variety of microbial hosts. Expression of the toxin gene results, directly or indirectly, in the intracellular production and maintenance of the pesticide. With suitable hosts, e.g., Pseudomonas, the microbes can be applied to the situs of mites where they will proliferate and be ingested by the mites. The result is a control of the mites. Alternatively, the microbe hosting the toxin gene can be treated under conditions that prolong the activity of the toxin produced in the cell. The treated cell then can be applied to the environment of the target pest. The resulting product retains the toxicity of the B.t. toxin.

Where the B.t. toxin gene is introduced via a suitable vector into a microbial host, and said host is applied to the environment in a living state, it is essential that certain host microbes be used. Microorganism hosts are selected which are known to occupy the "phytosphere" (phylloplane, phyllosphere, rhizosphere, and/or rhizoplane) of one or more crops of interest. These microorganisms are selected so as to be capable of successfully competing in the particular environment (crop and other insect habitats) with the wild-type microorganisms, provide for stable maintenance and expression of the gene expressing the polypeptide pesticide, and, desirably, provide for improved protection of the pesticide from environmental degradation and inactivation.

A large number of microorganisms are known to inhabit the phylloplane (the surface of the plant leaves) and/or the rhizosphere (the soil surrounding plant roots). These microorganisms include bacteria, algae, and fungi. Of particular interest are microorganisms, such as bacteria, e.g., genera Bacillus, Pseudomonas, Erwinia, Serratia, Klebsiella, Xanthomonas, Streptomyces, Rhizobium, Rhodospseudomonas, Methylophilus, Agrobacterium, Acetobacter, Lactobacillus, Arthrobacter, Azotobacter, Leuconostoc, Alcaligenes and Clostridium; fungi, particularly yeast, e.g., genera Saccharomyces, Cryptococcus, Kluyveromyces, Sporobolomyces, Rhodotorula, and Aureobasidium; microalgae, e.g., families Cyanophyceae, Prochlorophyceae, Rhodophyceae, Dinophyceae, Chrysophyceae, Prymnesiophyceae, Xanthophyceae, Raphidophyceae, Bacillariophyceae, Eustigmatophyceae, Cryptophyceae, Euglenophyceae, Prasinophyceae, and Chlorophyceae. Of particular interest are such phytosphere bacterial species as Pseudomonas syringae, Pseudomonas fluorescens, Serratia marcescens, Acetobacter xylinum, Agrobacterium tumefaciens, Rhodospseudomonas spheroides, Xanthomonas campestris, Rhizobium melioli, Alcaligenes entrophus, and Azotobacter vinlandii; and phytosphere yeast species such as Rhodotorula rubra, R. glutinis, R. marina, R.

aurantiaca, Cryptococcus albidus, C. diffluens, C. laurentii, Saccharomyces rosei, S. pretoriensis, S. cerevisiae, Sporobolomyces roseus, S. odoratus, Cluyveromyces veronae, and Aureobasidium pollulans. Of particular interest are the pigmented microorganisms.

5 A wide variety of ways are available for introducing a B.t. gene expressing a toxin into the microorganism host under conditions which allow for stable maintenance and expression of the gene. One can provide for DNA constructs which include the transcriptional and translational regulatory signals for expression of the toxin gene, the toxin gene under their regulatory control and a DNA sequence homologous with a sequence in the host organism, whereby integration will occur, and/or a replication system which is functional in the host, 10 whereby integration or stable maintenance will occur.

The transcriptional initiation signals will include a promoter and a transcriptional initiation start site. In some instances, it may be desirable to provide for regulative expression of the toxin, where expression of the toxin will only occur after release into the environment. This can be achieved with operators or a region binding to an activator or enhancers, which 15 are capable of induction upon a change in the physical or chemical environment of the microorganisms. For example, a temperature sensitive regulatory region may be employed, where the organisms may be grown up in the laboratory without expression of a toxin, but upon release into the environment, expression would begin. Other techniques may employ a specific nutrient medium in the laboratory, which inhibits the expression of the toxin, where 20 the nutrient medium in the environment would allow for expression of the toxin. For translational initiation, a ribosomal binding site and an initiation codon will be present.

Various manipulations may be employed for enhancing the expression of the messenger RNA, particularly by using an active promoter, as well as by employing sequences, which enhance the stability of the messenger RNA. The transcriptional and translational 25 termination region will involve stop codon(s), a terminator region, and optionally, a polyadenylation signal. A hydrophobic "leader" sequence may be employed at the amino terminus of the translated polypeptide sequence in order to promote secretion of the protein across the inner membrane.

In the direction of transcription, namely in the 5' to 3' direction of the coding or sense sequence, the construct will involve the transcriptional regulatory region, if any, and 30 the promoter, where the regulatory region may be either 5' or 3' of the promoter, the ribosomal binding site, the initiation codon, the structural gene having an open reading frame in phase with the initiation codon, the stop codon(s), the polyadenylation signal sequence, if any, and the terminator region. This sequence as a double strand may be used by itself for transformation of a microorganism host, but will usually be included with a DNA sequence 35 involving a marker, where the second DNA sequence may be joined to the toxin expression construct during introduction of the DNA into the host.

By a marker is intended a structural gene which provides for selection of those hosts which have been modified or transformed. The marker will normally provide for

selective advantage, for example, providing for biocide resistance, e.g., resistance to antibiotics or heavy metals; complementation, so as to provide prototrophy to an auxotrophic host, or the like. Preferably, complementation is employed, so that the modified host may not only be selected, but may also be competitive in the field. One or more markers may be employed in the development of the constructs, as well as for modifying the host. The organisms may be further modified by providing for a competitive advantage against other wild-type microorganisms in the field. For example, genes expressing metal chelating agents, e.g., siderophores, may be introduced into the host along with the structural gene expressing the toxin. In this manner, the enhanced expression of a siderophore may provide for a competitive advantage for the toxin-producing host, so that it may effectively compete with the wild-type microorganisms and stably occupy a niche in the environment.

Where no functional replication system is present, the construct will also include a sequence of at least 50 basepairs (bp), preferably at least about 100 bp, and usually not more than about 5000 bp of a sequence homologous with a sequence in the host. In this way, the probability of legitimate recombination is enhanced, so that the gene will be integrated into the host and stably maintained by the host. Desirably, the toxin gene will be in close proximity to the gene providing for complementation as well as the gene providing for the competitive advantage. Therefore, in the event that a toxin gene is lost, the resulting organism will be likely to also lose the complementing gene and/or the gene providing for the competitive advantage, so that it will be unable to compete in the environment with the gene retaining the intact construct.

A large number of transcriptional regulatory regions are available from a wide variety of microorganism hosts, such as bacteria, bacteriophage, cyanobacteria, algae, fungi, and the like. Various transcriptional regulatory regions include the regions associated with the trp gene, lac gene, gal gene, the lambda left and right promoters, the tac promoter, the naturally-occurring promoters associated with the toxin gene, where functional in the host. See for example, U.S. Patent Nos. 4,332,898, 4,342,832 and 4,356,270. The termination region may be the termination region normally associated with the transcriptional initiation region or a different transcriptional initiation region, so long as the two regions are compatible and functional in the host.

Where stable episomal maintenance or integration is desired, a plasmid will be employed which has a replication system which is functional in the host. The replication system may be derived from the chromosome, an episomal element normally present in the host or a different host, or a replication system from a virus which is stable in the host. A large number of plasmids are available, such as pBR322, pACYC184, RSF1010, pRO1614, and the like. See for example, Olson et al., (1982) J. Bacteriol. 150:6069, and Bagdasarian et al., (1981) Gene 16:237, and U.S. Patent Nos. 4,356,270, 4,362,817, and 4,371,625.

The B.t. gene can be introduced between the transcriptional and translational initiation region and the transcriptional and translational termination region, so as to be under

the regulatory control of the initiation region. This construct will be included in a plasmid, which will include at least one replication system, but may include more than one, where one replication system is employed for cloning during the development of the plasmid and the second replication system is necessary for functioning in the ultimate host. In addition, one or more markers may be present, which have been described previously. Where integration is desired, the plasmid will desirably include a sequence homologous with the host genome.

The transformants can be isolated in accordance with conventional ways, usually employing a selection technique, which allows for selection of the desired organism as against unmodified organisms or transferring organisms, when present. The transformants then can be tested for pesticidal activity.

Suitable host cells, where the pesticide-containing cells will be treated to prolong the activity of the toxin in the cell when the then treated cell is applied to the environment of target pest(s), may include either prokaryotes or eukaryotes, normally being limited to those cells which do not produce substances toxic to higher organisms, such as mammals. However, organisms which produce substances toxic to higher organisms could be used, where the toxin is unstable or the level of application sufficiently low as to avoid any possibility of toxicity to a mammalian host. As hosts, of particular interest will be the prokaryotes and the lower eukaryotes, such as fungi, as disclosed previously.

Characteristics of particular interest in selecting a host cell for purposes of production include ease of introducing the B.t. gene into the host, availability of expression systems, efficiency of expression, stability of the pesticide in the host, and the presence of auxiliary genetic capabilities. Characteristics of interest for use as a pesticide microcapsule include protective qualities for the pesticide, such as thick cell walls, pigmentation, and intracellular packaging or formation of inclusion bodies; survival in aqueous environments; lack of mammalian toxicity; attractiveness to pests for ingestion; ease of killing and fixing without damage to the toxin; and the like. Other considerations include ease of formulation and handling, economics, storage stability, and the like.

The cell will usually be intact and be substantially in the proliferative form when treated, rather than in a spore form, although in some instances spores may be employed.

Treatment of the microbial cell, e.g., a microbe containing the B.t. toxin gene, can be by chemical or physical means, or by a combination of chemical and/or physical means, so long as the technique does not deleteriously affect the properties of the toxin, nor diminish the cellular capability in protecting the toxin. Examples of chemical reagents are halogenating agents, particularly halogens of atomic no. 17-80. More particularly, iodine can be used under mild conditions and for sufficient time to achieve the desired results. Other suitable techniques include treatment with aldehydes, such as formaldehyde and glutaraldehyde; anti-infectives, such as zephiran chloride and cetylpyridinium chloride; alcohols, such as isopropyl and ethanol; various histologic fixatives, such as Lugol iodine, Bouin's fixative, and Helly's fixative (See: Humason, Gretchen L., Animal Tissue Techniques, W.H. Freeman and

Company, 1967); or a combination of physical (heat) and chemical agents that preserve and prolong the activity of the toxin produced in the cell when the cell is administered to the host animal. Examples of physical means are short wavelength radiation such as gamma-radiation and X-radiation, freezing, UV irradiation, lyophilization, and the like.

5 The cells generally will have enhanced structural stability which will enhance resistance to environmental conditions. Where the pesticide is in a proform, the method of inactivation should be selected so as not to inhibit processing of the proform to the mature form of the pesticide by the target pest pathogen. For example, formaldehyde will crosslink proteins and could inhibit processing of the proform of a polypeptide pesticide. The method of inactivation or killing retains at least a substantial portion of the bio-availability or
10 bioactivity of the toxin.

 The cellular host containing the B.t. insecticidal gene may be grown in any convenient nutrient medium, where the DNA construct provides a selective advantage, providing for a selective medium so that substantially all or all of the cells retain the B.t. gene.
15 These cells may then be harvested in accordance with conventional ways. Alternatively, the cells can be treated prior to harvesting.

 The B.t. cells of the invention can be cultured using standard art media and fermentation techniques. Upon completion of the fermentation cycle the bacteria can be harvested by first separating the B.t. spores and crystals from the fermentation broth by means
20 well known in the art. The recovered B.t. spores and crystals can be formulated into a wettable powder, liquid concentrate, granules or other formulations by the addition of surfactants, dispersants, inert carriers, and other components to facilitate handling and application for particular target pests. These formulations and application procedures are all well known in the art.

25 Formulated bait granules containing an attractant and spores and crystals of the B.t. isolates, or recombinant microbes comprising the gene(s) obtainable from the B.t. isolates disclosed herein, can be applied to the soil or in the vicinity of stored products. Formulated product can also be applied as a seed-coating or root treatment or total plant treatment at later stages of the crop cycle.

30 Mutants of the novel isolates of the invention can be made by procedures well known in the art. For example, an asporogenous mutant can be obtained through ethylmethane sulfonate (EMS) mutagenesis of a novel isolate. The mutants can be made using ultraviolet light and nitrosoguanidine by procedures well known in the art.

35 A smaller percentage of the asporogenous mutants will remain intact and not lyse for extended fermentation periods; these strains are designated lysis minus (-). Lysis minus strains can be identified by screening asporogenous mutants in shake flask media and selecting those mutants that are still intact and contain toxin crystals at the end of the fermentation. Lysis minus strains are suitable for a cell fixation process that will yield a protected, encapsulated toxin protein.

To prepare a phage resistant variant of said asporogenous mutant, an aliquot of the phage lysate is spread onto nutrient agar and allowed to dry. An aliquot of the phage sensitive bacterial strain is then plated directly over the dried lysate and allowed to dry. The plates are incubated at 30°C. The plates are incubated for 2 days and, at that time, numerous colonies could be seen growing on the agar. Some of these colonies are picked and subcultured onto nutrient agar plates. These apparent resistant cultures are tested for resistance by cross streaking with the phage lysate. A line of the phage lysate is streaked on the plate and allowed to dry. The presumptive resistant cultures are then streaked across the phage line. Resistant bacterial cultures show no lysis anywhere in the streak across the phage line after overnight incubation at 30°C. The resistance to phage is then reconfirmed by plating a lawn of the resistant culture onto a nutrient agar plate. The sensitive strain is also plated in the same manner to serve as the positive control. After drying, a drop of the phage lysate is plated in the center of the plate and allowed to dry. Resistant cultures showed no lysis in the area where the phage lysate has been placed after incubation at 30°C for 24 hours.

Following are examples which illustrate procedures, including the best mode, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

Example 1 — Culturing of the B.t. Isolates

A subculture of the B.t. isolates, or mutants thereof, can be used to inoculate the following medium, a peptone, glucose, salts medium.

	Bacto Peptone	7.5 g/l
	Glucose	1.0 g/l
	KH ₂ PO ₄	3.4 g/l
	K ₂ HPO ₄	4.35 g/l
	Salt Solution	5.0 ml/l
	CaCl ₂ Solution	5.0 ml/l
	pH 7.2	
	Salts Solution (100 ml)	
	MgSO ₄ ·7H ₂ O	2.46 g
	MnSO ₄ ·H ₂ O	0.04 g
	ZnSO ₄ ·7H ₂ O	0.28 g
	FeSO ₄ ·7H ₂ O	0.40 g
	CaCl ₂ Solution (100 ml)	
	CaCl ₂ ·2H ₂ O	3.66 g

The salts solution and CaCl_2 solution are filter-sterilized and added to the autoclaved and cooked broth at the time of inoculation. Flasks are incubated at 30°C on a rotary shaker at 200 rpm for 64 hr.

The above procedure can be readily scaled up to large fermentors by procedures well known in the art.

The B.t. spores and/or crystals, obtained in the above fermentation, can be isolated by procedures well known in the art. A frequently-used procedure is to subject the harvested fermentation broth to separation techniques, e.g., centrifugation.

Example 2 – Purification of Protein and Amino Acid Sequencing

The B.t. isolates PS17, PS52A1 and PS69D1 were cultured as described in Example

1. The parasporal inclusion bodies were partially purified by sodium bromide (28-38%) isopycnic gradient centrifugation (Pfannenstiel, M.A., E.J. Ross, V.C. Kramer, and K.W. Nickerson [1984] FEMS Microbiol. Lett. 21:39). The proteins were bound to PVDF membranes (Millipore, Bedford, MA) by western blotting techniques (Towbin, H., T. Staehelin, and K. Gordon [1979] Proc. Natl. Acad. Sci. USA 76:4350) and the N-terminal amino acid sequences were determined by the standard Edman reaction with an automated gas-phase sequenator (Hunkapiller, M.W., R.M. Hewick, W.L. Dreyer, and L.E. Hood [1983] Meth. Enzymol. 91:399). The sequences obtained were:

PS17a: A I L N E L Y P S V P Y N V (SEQ ID NO. 12)

PS17b: A I L N E L Y P S V P Y N V (SEQ ID NO. 13)

PS52A1: M I I D S K T T L P R H S L I N T (SEQ ID NO. 14)

PS69D1: M I L G N G K T L P K H I R L A H I F A T Q N S (SEQ ID NO. 15)

Example 3 – Cloning of Novel Toxin Genes and Transformation into Escherichia coli

Total cellular DNA was prepared by growing the cells B.t. PS17 to a low optical density ($\text{OD}_{600} = 1.0$) and recovering the cells by centrifugation. The cells were protoplasted in TES buffer (30 mM Tris-Cl, 10 mM EDTA, 50 mM NaCl, pH = 8.0) containing 20 % sucrose and 50 mg/ml lysozyme. The protoplasts were lysed by addition of SDS to a final concentration of 4%. The cellular material was precipitated overnight at 4°C in 100 mM (final concentration) neutral potassium chloride. The supernate was extracted twice with phenol/chloroform (1:1). The DNA was precipitated with ethanol and purified by isopycnic banding on a cesium chloride-ethidium bromide gradient.

Total cellular DNA from PS17 was digested with EcoRI and separated by electrophoresis on a 0.8% (w/v) Agarose-TAE (50 mM Tris-HCl, 20 mM NaOAc, 2.5 mM EDTA, pH=8.0) buffered gel. A Southern blot of the gel was hybridized with a [^{32}P] - radiolabeled oligonucleotide probe derived from the N-terminal amino acid sequence of purified 130 kDa protein from PS17. The sequence of the oligonucleotide synthesized is (GCAATTTTAAATGAATTATATCC) (SEQ ID NO. 16). Results showed that the

hybridizing EcoRI fragments of PS17 are 5.0 kb, 4.5 kb, 2.7 kb and 1.8 kb in size, presumptively identifying at least four new acaride-active toxin genes, PS17d, PS17b, PS17a and PS17e, respectively.

5 A library was constructed from PS17 total cellular DNA partially digested with Sau3A and size fractionated by electrophoresis. The 9 to 23 kb region of the gel was excised and the DNA was electroeluted and then concentrated using an ElutipTM ion exchange column (Schleicher and Schuel, Keene NH). The isolated Sau3A fragments were ligated into LambdaGEM-11TM (PROMEGA). The packaged phage were plated on KW251 E. coli cells (PROMEGA) at a high titer and screened using the above radiolabeled synthetic
10 oligonucleotide as a nucleic acid hybridization probe. Hybridizing plaques were purified and rescreened at a lower plaque density. Single isolated purified plaques that hybridized with the probe were used to infect KW251 E. coli cells in liquid culture for preparation of phage for DNA isolation. DNA was isolated by standard procedures.

15 Recovered recombinant phage DNA was digested with EcoRI and separated by electrophoresis on a 0.8% agarose-TAE gel. The gel was Southern blotted and hybridized with the oligonucleotide probe to characterize the toxin genes isolated from the lambda library. Two patterns were present, clones containing the 4.5 kb (PS17b) or the 2.7 kb (PS17a) EcoRI fragments. Preparative amounts of phage DNA were digested with SalI (to release the inserted DNA from lambda arms) and separated by electrophoresis on a 0.6% agarose-TAE gel. The
20 large fragments, electroeluted and concentrated as described above, were ligated to SalI-digested and dephosphorylated pBClac, an E. coli/B.t. shuttle vector comprised of replication origins from pBC16 and pUC19. The ligation mix was introduced by transformation into NM522 competent E. coli cells and plated on LB agar containing ampicillin, isopropyl-(Beta)-D-thiogalactoside (IPTG) and 5-Bromo-4-Chloro-3-indolyl-(Beta)-D-galactoside (XGAL).
25 White colonies, with putative insertions in the (Beta)-galactosidase gene of pBClac, were subjected to standard rapid plasmid purification procedures to isolate the desired plasmids. The selected plasmid containing the 2.7 kb EcoRI fragment was named pMYC1627 and the plasmid containing the 4.5 kb EcoRI fragment was called pMYC1628.

30 The toxin genes were sequenced by the standard Sanger dideoxy chain termination method using the synthetic oligonucleotide probe, disclosed above, and by "walking" with primers made to the sequence of the new toxin genes.

The PS17 toxin genes were subcloned into the shuttle vector pHT3101 (Lereclus, D. et al. [1989] FEMS Microbiol. Lett. 60:211-218) using standard methods for expression in B.t. Briefly, SalI fragments containing the 17a and 17b toxin genes were isolated from
35 pMYC1629 and pMYC1627, respectively, by preparative agarose gel electrophoresis, electroelution, and concentrated, as described above. These concentrated fragments were ligated into SalI-cleaved and dephosphorylated pHT3101. The ligation mixtures were used separately to transform frozen, competent E. coli NM522. Plasmids from each respective recombinant E. coli strain were prepared by alkaline lysis and analyzed by agarose gel

electrophoresis. The resulting subclones, pMYC2311 and pMYC2309, harbored the 17a and 17b toxin genes, respectively. These plasmids were transformed into the acrySTALLIFEROUS B.t. strain, HD-1 cryB (Aronson, A., Purdue University, West Lafayette, IN), by standard electroporation techniques (Instruction Manual, Biorad, Richmond, CA).

5 Recombinant B.t. strains HD-1 cryB [pMYC2311] and [pMYC2309] were grown to sporulation and the proteins purified by NaBr gradient centrifugation as described above for the wild-type B.t. proteins.

10 Example 4 – Molecular Cloning of Gene Encoding a Novel Toxin From *Bacillus thuringiensis* strain PS52A1

Total cellular DNA was prepared from *Bacillus thuringiensis* PS52A1 (B.t. PS52A1) as disclosed in Example 3.

RFLP analyses were performed by standard hybridization of Southern blots of PS52A1 DNA with a ³²P-labeled oligonucleotide probe designed from the N-terminal amino acid sequence disclosed in Example 2. The sequence of this probe is:

15 5' ATG ATT ATT GAT TCT AAA ACA ACA TTA CCA AGA CAT TCA/T
TTA ATA/T AAT ACA/T ATA/T AA 3' (SEQ ID NO. 17)

This probe was designated 52A1-C. Hybridizing bands included an approximately 3.6 kbp *HindIII* fragment and an approximately 8.6 kbp *EcoRV* fragment. A gene library was constructed from PS52A1 DNA partially digested with *Sau3A*. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 6.6 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, and recovered by ethanol precipitation after purification on an Elutip-D ion exchange column. The *Sau3A* inserts were ligated into *BamHI*-digested LambdaGem-11 (Promega). Recombinant phage were packaged and plated on *E. coli* KW251 cells (Promega). Plaques were screened by hybridization with the radiolabeled 52A1-C oligonucleotide probe disclosed above. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of phage DNA by standard procedures (Maniatis et al.). For subcloning, preparative amounts of DNA were digested with *EcoRI* and *SalI*, and electrophoresed on an agarose gel. The approximately 3.1 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as above. The purified DNA insert was ligated into *EcoRI* + *SalI*-digested pHTBlueII (an *E. coli/B. thuringiensis* shuttle vector comprised of pBluescript S/K [Stratagene] and the replication origin from a resident B.t. plasmid [D. Lereclus et al. 1989. FEMS Microbiology Letters 60:211-218]). The ligation mix was used to transform frozen, competent *E. coli* NM522 cells (ATCC 47000). Transformants were plated on LB agar containing ampicillin, isopropyl-(Beta)-D-thiogalactoside (IPTG), and 5-Bromo-4-Chloro-3-indolyl-(Beta)-D-galactoside (XGAL). Plasmids were purified from putative recombinants by alkaline lysis (Maniatis et al.) and analyzed by electrophoresis of *EcoRI* and

SaII digests on agarose gels. The desired plasmid construct, pMYC2321 contains a toxin gene that is novel compared to the maps of other toxin genes encoding acaricidal proteins.

Plasmid pMYC2321 was introduced into an acrySTALLIFEROUS (Cry⁻) *B.t.* host by electroporation. Expression of an approximately 55-60 kDa crystal protein was verified by SDS-PAGE analysis.

Example 5 – Molecular Cloning of Gene Encoding a Novel Toxin From *Bacillus Thuringiensis* strain PS69D1

Total cellular DNA was prepared from PS69D1 (*B.t.* PS69D1) as disclosed in Example 3. RFLP analyses were performed by standard hybridization of Southern blots of PS69D1 DNA with a 32P-labeled oligonucleotide probe designated as 69D1-D. The sequence of the 69D1-D probe was:

5' AAA CAT ATT AGA TTA GCA CAT ATT TTT GCA ACA CAA
AA 3' (SEQ ID NO. 18)

Hybridizing bands included an approximately 2.0 kbp *HindIII* fragment.

A gene library was constructed from PS69D1 DNA partially digested with *Sau3A*. Partial restriction digests were fractionated by agarose gel electrophoresis. DNA fragments 6.6 to 23 kbp in size were excised from the gel, electroeluted from the gel slice, and recovered by ethanol precipitation after purification on an Elutip-D ion exchange column. The *Sau3A* inserts were ligated into *Bam*HI-digested LambdaGem-11 (Promega, Madison, WI). Recombinant phage were packaged and plated on *E. coli* KW251 cells (Promega, Madison, WI). Plaques were screened by hybridization with the radiolabeled 69D1-D oligonucleotide probe. Hybridizing phage were plaque-purified and used to infect liquid cultures of *E. coli* KW251 cells for isolation of phage DNA by standard procedures (Maniatis et al. [1982] *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY). For subcloning, preparative amounts of DNA were digested with *HindIII* and electrophoresed on an agarose gel. The approximately, 2.0 kbp band containing the toxin gene was excised from the gel, electroeluted from the gel slice, and purified by ion exchange chromatography as above. The purified DNA insert was ligated into *HindIII*-digested pHTBlueII (and *E. coli/B.t.* shuttle vector comprised of pBluescript S/K (Stratagene, San Diego, CA) and the replication origin from a resident *B.t.* plasmid (D. Lereclus et al [1989] FEMS Microbiol. Lett. 60:211-218). The ligation mix was used to transform frozen, competent *E. coli* NM522 cells (ATCC 47000). Transformants were plated on LB agar containing 5-bromo-4-chloro-3-indolyl-(Beta)-D-galactoside (XGAL). Plasmids were purified from putative recombinants by alkaline lysis (Maniatis et al., *supra*) and analyzed by electrophoresis of *HindIII* digests on agarose gels. The desired plasmid construct, pMYC2317, contains a toxin gene that is novel compared to the maps of other toxin genes encoding insecticidal proteins.

Example 6 -- Activity of B.t. Isolates Against Mites

B. thuringiensis isolates of the invention were tested as spray-dried powders of fermentation broths which were concentrated by centrifugation. Pellets, which consist of water and biomass (spores, crystalline delta-endotoxins, cellular debris and growth media) were mixed with a standard carrier, preservative and surfactant. Powders, which consisted of 25% biomass, were made using a Yamato spray drier. (Sold by Yamato Scientific Co., Ltd. Tokyo, Japan)

All broths were tested for the presence of beta-exotoxin by a larval house fly bioassay (Campbell, D.P., Dieball, D.E. and Brackett, J.M., 1987, Rapid HPLC assay for the β -exotoxin of Bacillus thuringiensis. J. Agric. Food Chem. 35:156-158). Only isolates which tested free of β -exotoxin were used in the assays against mites.

B. thuringiensis isolates were tested using an artificial feeding assay. Spray-dried powders were prepared for testing by mixing 25mg of powder in 5 ml of a 10% sucrose solution. This mixture was then sonicated for 8 min to produce a suspension.

Two ml of suspension was placed in a reservoir consisting of a metal ring with a Parafilm™ M film bottom. A petri dish containing approximately 30 female Two-spotted spider mites (Tetranychus urticae) was placed on the underside of the film. Mites were allowed to feed on the sucrose solution for 24 hrs and then transferred to 2 cm French bean leaf discs (20 mites per disc). Mortality was determined after 7 days (Table 2). Each assay was done in triplicate.

TABLE 2. Toxicity of Bacillus thuringiensis isolates to the two spotted spider mite, Tetranychus urticae. Mortality was determined after 7 days of treatment.

Isolate	Percent Mortality
<u>B.t.</u> PS50C	63
<u>B.t.</u> PS86A1	85
<u>B.t.</u> PS69D1	77
<u>B.t.</u> PS72L1	85
<u>B.t.</u> PS75J1	85
<u>B.t.</u> PS83E5	70
<u>B.t.</u> PS45B1	82
<u>B.t.</u> PS24J	90
<u>B.t.</u> PS94R3	97
<u>B.t.</u> PS17	>90
<u>B.t.</u> PS62B1	>90
<u>B.t.</u> PS74G1	>90
Control	10

Example 7 - Cloning of Novel Acaricide-Active Genes Using Generic Oligonucleotide Primers

The acaricidal gene of a new acaricidal *B.t.* isolate can be obtained from DNA of the strain by performing the standard polymerase chain reaction using the oligonucleotides of SEQ ID NO. 21 or SEQ ID NO. 20 as reverse primers and SEQ ID NO. 10, SEQ ID NO. 11, SEQ ID NO. 16, Probe B of SEQ ID NO. 5 (AAT GAA GTAT TAT CCA/T GTA/T AAT), or SEQ ID NO. 19 as forward primers. The expected PCR fragments would be approximately 330 to 600 bp (with either reverse primer and SEQ ID NO. 10), 1000 to 1400 bp (with either reverse primer and SEQ ID NO. 11), and 1800 to 2100 bp (with either reverse primer and any of the three N-terminal primers, SEQ ID NO. 5 (Probe B), SEQ ID NO. 16, and SEQ ID NO. 19). Alternatively, a complement from the primer family described by SEQ ID NO. 10 can be used as reverse primer with SEQ ID NO. 11, SEQ ID NO. 16, SEQ ID NO. 5 (Probe B), or SEQ ID NO. 19 as forward primers. The expected PCR fragments would be approximately 650 to 1000 bp with SEQ ID NO. 11, and 1400 to 1800 bp (for the three N-terminal primers, SEQ ID NO. 5 (Probe B), SEQ ID NO. 16, and SEQ ID NO. 19). Amplified DNA fragments of the indicated sizes can be radiolabeled and used as probes to clone the entire gene.

Example 8 - Further Cloning of Novel Acaricide-Active Genes Using Generic Oligonucleotide Primers

A gene coding for a acaricidal toxin of an acaricidal *B.t.* isolate can also be obtained from DNA of the strain by performing the standard polymerase chain reaction using oligonucleotides derived from the PS52A1 and PS69D1 gene sequences as follows:

1. Forward primer "TGATTTT(T or A)(C or A)TCAATTATAT(A or G)A(G or T)GTTTAT" (SEQ ID NO. 22) can be used with primers complementary to probe "AAGAGTTA(C or T)TA(A or G)A(G or A)AAAGTA" (SEQ ID NO. 23), probe "TTAGGACCATT(A or G)(C or T)T(T or A)GGATTTGTTGT(A or T)TATGAAAT" (SEQ ID NO. 24), and probe "GA(C or T)AGAGATGT(A or T)AAAAT(C or T)(T or A)TAGGAATG" (SEQ ID NO. 25) to produce amplified fragments of approximately 440, 540, and 650 bp, respectively.

2. Forward primer "TT(A or C)TTAAA(A or T)C(A or T)GCTAATGATATT" (SEQ ID NO. 26) can be used with primers complementary to SEQ ID NO. 23, SEQ ID NO. 24, and SEQ ID NO. 25 to produce amplified fragments of approximately 360, 460, and 570 bp, respectively.

3. Forward primer SEQ ID NO. 23 can be used with primers complementary to SEQ ID NO. 24 and SEQ ID NO. 25 to produce amplified fragments of approximately 100 and 215 bp, respectively.

Amplified DNA fragments of the indicated sizes can be radiolabeled and used as probes to clone the entire gene.

Example 9 -- Insertion of Toxin Genes Into Plants

One aspect of the subject invention is the transformation of plants with genes coding for a acaricidal toxin. The transformed plants are resistant to attack by acarides.

Genes coding for acaricidal toxins, as disclosed herein, can be inserted into plant cells using a variety of techniques which are well known in the art. For example, a large number of cloning vectors comprising a replication system in *E. coli* and a marker that permits selection of the transformed cells are available for preparation for the insertion of foreign genes into higher plants. The vectors comprise, for example, pBR322, pUC series, M13mp series, pACYC184, etc. Accordingly, the sequence coding for the B.t. toxin can be inserted into the vector at a suitable restriction site. The resulting plasmid is used for transformation into *E. coli*. The *E. coli* cells are cultivated in a suitable nutrient medium, then harvested and lysed. The plasmid is recovered. Sequence analysis, restriction analysis, electrophoresis, and other biochemical-molecular biological methods are generally carried out as methods of analysis. After each manipulation, the DNA sequence used can be cleaved and joined to the next DNA sequence. Each plasmid sequence can be cloned in the same or other plasmids. Depending on the method of inserting desired genes into the plant, other DNA sequences may be necessary. If, for example, the Ti or Ri plasmid is used for the transformation of the plant cell, then at least the right border, but often the right and the left border of the Ti or Ri plasmid T-DNA, has to be joined as the flanking region of the genes to be inserted.

The use of T-DNA for the transformation of plant cells has been intensively researched and sufficiently described in EP 120 516; Hoekema (1985) In: The Binary Plant Vector System, Offset-drukkerij Kanters B.V., Alblasserdam, Chapter 5; Fraley et al., Crit. Rev. Plant Sci. 4:1-46; and An et al. (1985) EMBO J. 4:277-287.

Once the inserted DNA has been integrated in the genome, it is relatively stable there and, as a rule, does not come out again. It normally contains a selection marker that confers on the transformed plant cells resistance to a biocide or an antibiotic, such as kanamycin, G 418, bleomycin, hygromycin, or chloramphenicol, inter alia. The individually employed marker should accordingly permit the selection of transformed cells rather than cells that do not contain the inserted DNA.

A large number of techniques are available for inserting DNA into a plant host cell. Those techniques include transformation with T-DNA using *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* as transformation agent, fusion, injection, or electroporation as well as other possible methods. If agrobacteria are used for the transformation, the DNA to be inserted has to be cloned into special plasmids, namely either into an intermediate vector or into a binary vector. The intermediate vectors can be integrated into the Ti or Ri plasmid by homologous recombination owing to sequences that are homologous to sequences in the T-DNA. The Ti or Ri plasmid also comprises the vir region necessary for the transfer of the

T-DNA. Intermediate vectors cannot replicate themselves in agrobacteria. The intermediate vector can be transferred into *Agrobacterium tumefaciens* by means of a helper plasmid (conjugation). Binary vectors can replicate themselves both in *E. coli* and in agrobacteria. They comprise a selection marker gene and a linker or polylinker which are framed by the right and left T-DNA border regions. They can be transformed directly into agrobacteria (Holsters et al. [1978] *Mol. Gen. Genet.* 163:181-187). The agrobacterium used as host cell is to comprise a plasmid carrying a vir region. The vir region is necessary for the transfer of the T-DNA into the plant cell. Additional T-DNA may be contained. The bacterium so transformed is used for the transformation of plant cells. Plant explants can advantageously be cultivated with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* for the transfer of the DNA into the plant cell. Whole plants can then be regenerated from the infected plant material (for example, pieces of leaf, segments of stalk, roots, but also protoplasts or suspension-cultivated cells) in a suitable medium, which may contain antibiotics or biocides for selection. The plants so obtained can then be tested for the presence of the inserted DNA. No special demands are made of the plasmids in the case of injection and electroporation. It is possible to use ordinary plasmids, such as, for example, pUC derivatives.

The transformed cells grow inside the plants in the usual manner. They can form germ cells and transmit the transformed trait(s) to progeny plants. Such plants can be grown in the normal manner and crossed with plants that have the same transformed hereditary factors or other hereditary factors. The resulting hybrid individuals have the corresponding phenotypic properties.

Example 10 – Cloning of *Bacillus thuringiensis* Genes Into Baculoviruses

The genes coding for the insecticidal toxins, as disclosed herein, can be cloned into baculoviruses such as *Autographa californica* nuclear polyhedrosis virus (AcNPV). Plasmids can be constructed that contain the AcNPV genome cloned into a commercial cloning vector such as pUC8. The AcNPV genome is modified so that the coding region of the polyhedrin gene is removed and a unique cloning site for a passenger gene is placed directly behind the polyhedrin promoter. Examples of such vectors are pGP-B6874, described by Pennock et al. (Pennock, G.D., Shoemaker, C. and Miller, L.K. [1984] *Mol. Cell. Biol.* 4:399-406), and pAC380, described by Smith et al. (Smith, G.E., Summers, M.D. and Fraser, M.J. [1983] *Mol. Cell. Biol.* 3:2156-2165). The genes coding for the protein toxins of the invention can be modified with BamHI linkers at appropriate regions both upstream and downstream from the coding region and inserted into the passenger site of one of the AcNPV vectors.

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Payne, Jewel M.
Cannon, Raymond J.C.
Bagley, Angela L.
- (ii) TITLE OF INVENTION: Novel *Bacillus thuringiensis* Isolates for Controlling Acarides
- (iii) NUMBER OF SEQUENCES: 30
- (iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: David R. Saliwanchik
(B) STREET: 2421 N.W. 41st Street, Suite A-1
(C) CITY: Gainesville
(D) STATE: FL
(E) COUNTRY: USA
(F) ZIP: 32606
- (v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC Compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:
- (vii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Saliwanchik, David R.
(B) REGISTRATION NUMBER: 31,794
(C) REFERENCE/DOCKET NUMBER: M/S 104
- (ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 904-375-8100
(B) TELEFAX: 904-372-5800

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4155 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) ORIGINAL SOURCE:
(A) ORGANISM: *Bacillus thuringiensis*
(B) STRAIN: PS17
(C) INDIVIDUAL ISOLATE: PS17a
- (vi) IMMEDIATE SOURCE:
(B) CLONE: E. coli NM522(pMYC 1627) NRRL B-18651

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGTTTAAGTT TAATTACATT AGCTGTACCG GAAATGGTA TTTTACACC TTTCATCGGT	300
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CAAACATTTT TAAATGGGGA AATAAGTGGT TTACAAAATT TAGCAGCAAG ATACCAGTCT	480
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(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1385 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: BACILLUS THURINGIENSIS
 - (C) INDIVIDUAL ISOLATE: PS17
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC 1627) NRRL B-18651

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Gly Leu Ser Leu Ile Thr Leu Ala Val Pro Glu Ile Gly Ile Phe Thr
85     90     95
Pro Phe Ile Gly Leu Phe Phe Ala Ala Leu Asn Lys His Asp Ala Pro
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145    150    155    160

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 Thr Leu 210 Leu Gly Leu Pro Tyr 215 Tyr Ala Ile Leu Ala 220 Ser Met His Leu
 Met 225 Leu Leu Arg Asp Ile 230 Ile Thr Lys Gly Pro 235 Thr Trp Asp Ser Lys 240
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 Asn Asn Ile Lys 260 Leu Tyr Ser Lys Thr 265 Ile Tyr Asp Val Phe 270 Gln Lys
 Gly Leu Ala 275 Ser Tyr Gly Thr Pro 280 Ser Asp Leu Glu Ser 285 Phe Ala Lys
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 Asp Ile Ser Leu Gln 325 Lys Thr Arg Arg Ile 330 Leu Ser Pro Phe 335 Ile Pro
 Ile Arg Thr Ala 340 Asp Gly Leu Thr Leu 345 Asn Asn Thr Ser Ile 350 Asp Thr
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 Lys Glu 370 Arg Ile Leu Lys Gln 375 Phe Lys Leu Tyr Pro 380 Ser Trp Arg Ala
 Gly Gln 385 Tyr Gly Gly Leu 390 Leu Gln Pro Tyr Leu 395 Trp Ala Ile Glu Val 400
 Gln Asp Ser Val Gln 405 Thr Arg Leu Tyr Gly 410 Gln Leu Pro Ala Val 415 Asp
 Pro Gln Ala Gly 420 Pro Asn Tyr Val Ser 425 Ile Asp Ser Ser Asn 430 Pro Ile
 Ile Gln Ile 435 Asn Met Asp Thr Trp 440 Lys Thr Pro Pro Gln 445 Gly Ala Ser
 Gly Trp 450 Asn Thr Asn Leu Met 455 Arg Gly Ser Val Ser 460 Gly Leu Ser Phe
 Leu 465 Gln Arg Asp Gly Thr 470 Arg Leu Ser Ala Gly 475 Met Gly Gly Gly Phe 480
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 Tyr Gly Thr Pro 500 Tyr Gln Thr Ser Asp 505 Asn Tyr Ser Gly His 510 Val Gly
 Ala Leu Val Gly 515 Val Ser Thr Pro 520 Gln Glu Ala Thr Leu 525 Pro Asn Ile
 Ile Gly 530 Gln Pro Asp Glu Gln 535 Gly Asn Val Ser Thr 540 Met Gly Phe Pro
 Phe 545 Glu Lys Ala Ser Tyr 550 Gly Gly Thr Val Val 555 Lys Glu Trp Leu Asn 560
 Gly Ala Asn Ala Met 565 Lys Leu Ser Pro Gly 570 Gln Ser Ile Gly Ile 575 Pro
 Ile Thr Asn Val 580 Thr Ser Gly Glu Tyr 585 Gln Ile Arg Cys Arg 590 Tyr Ala
 Ser Asn Asp 595 Asn Thr Asn Val Phe 600 Phe Asn Val Asp Thr 605 Gly Gly Ala
 Asn Pro 610 Ile Phe In In Ile 615 Asn Phe Ala Ser Thr 620 Val Asp Asn Asn

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 625 630 635 640
 Thr Thr Asp Asn Ser Phe Thr Glu Ile Pro Ala Lys Thr Ile Asn Val
 645 650 655
 His Leu Thr Asn Gln Gly Ser Ser Asp Val Phe Leu Asp Arg Ile Glu
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 675 680 685
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 690 695 700
 Tyr Asp Ile Ile Val Asn Gly Gln Ala Asn Ser Ser Ser Ile Ala Ser
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 Val Tyr Ser Pro Phe Asp Ile Thr Ile Gln Thr Glu Ala Asp Ser Leu
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 Ser Gly Thr Gln Asn Met Leu Ala His Asn Val Ser Asp His Asp Ile
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 930 935 940
 Asp Glu Lys Lys Ala Leu Arg Lys Leu Val Asn Gln Ala Lys Arg Leu
 945 950 955 960
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 965 970 975
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 980 985 990
 Phe Lys Ser Asp His Val Leu Leu Pro Pro Pro Gly Leu Ser Pro Ser
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 Tyr Ile Phe Gln Lys Val Glu Glu Ser Lys Leu Lys Pro Asn Thr Arg
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 1025 1030 1035 1040
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 1060 1065 1070
 Arg Ser Thr Ser Asn Gly Thr Leu Gly Asp Pro His Phe Ph s r Tyr
 1075 1080 1085

Ser Ile Asp Val Gly Ala Leu Asp Leu Gln Ala Asn Pro ly Ile Glu
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 Phe Gly Leu Arg Ile Val Asn Pro Thr Gly Met Ala Arg Val Ser Asn
 1105 1110 1115 1120
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 1170 1175 1180
 Tyr Gln Asn Ile Asp Ala Ile Val Leu Pro Thr Leu Pro Lys Leu Arg
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 His Trp Phe Met Ser Asp Arg Phe Ser Glu Gln Gly Asp Ile Met Ala
 1205 1210 1215
 Lys Phe Gln Gly Ala Leu Asn Arg Ala Tyr Ala Gln Leu Glu Gln Ser
 1220 1225 1230
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 Leu Arg Leu Pro Asp Trp Ser Ser Ser Val Ser Gln Met Ile Glu Ile
 1265 1270 1275 1280
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 1315 1320 1325
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 Asp Gly Glu Phe Leu Val Asp Asn Ile Ala Leu Val Glu Ala Pro Leu
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 Pro Thr Asp Asp Gln Asn Ser Glu Gly Asn Thr Ala Ser Ser Thr Asn
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(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 3867 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Bacillus thuringiensis*
 - (B) STRAIN: PS17
 - (C) INDIVIDUAL ISOLATE: PS17b
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC 1628) NRRL B-18652

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATGGCAATTT TAAATGAATT ATATCCATCT GTACCTTATA ATGTATTGGC GTATACGCCA 60
 CCTCTTTTT TACCTGATGC GGGTACACAA GCTACACCTG CTGACTTAAC AGCTTATGAA 120

CAATTGTTGA	AAAATTTAGA	AAAAGGGATA	AATGCTGGAA	CTTATTCGAA	AGCAATAGCT	180
GATGTACTTA	AAGGTATTTT	TATAGATGAT	ACAATAAATT	ATCAAACATA	TGTAAATATT	240
GGTTTAAGTT	TAATTACATT	AGCTGTACCG	GAAATTGGTA	TTTTTACACC	TTTCATCGGT	300
TTGTTTTTTG	CTGCATTGAA	TAAACATGAT	GCTCCACCTC	CTCCTAATGC	AAAAGATATA	360
TTTGAGGCTA	TGAAACCAGC	GATTCAAGAG	ATGATTGATA	GAACTTTAAC	TGCGGATGAG	420
CAAACATTTT	TAAATGGGGA	AATAAGTGGT	TTACAAAATT	TAGCAGCAAG	ATACCAGTCT	480
ACAAATGGATG	ATATTCAAAG	CCATGGAGGA	TTTAATAAGG	TAGATTCTGG	ATTAATTAAA	540
AAGTTTACAG	ATGAGGTACT	ATCTTTAAAT	AGTTTTTATA	CAGATCGTTT	ACCTGTATTT	600
ATTACAGATA	ATACAGCGGA	TGAACTTTG	TTAGGTCTTC	CTTATTATGC	TATACTTGCG	660
AGCATGCATC	TTATGTTATT	AAGAGATATC	ATTACTAAGG	GTCCGACATG	GGATTCTAAA	720
ATTAATTTCA	CACCAGATGC	AATTGATTCC	TTTAAAACCG	ATATTAAAAA	TAATATAAAG	780
CTTTACTCTA	AAACTATTTA	TGACGTATTT	CAGAAGGGAC	TTGCTTCATA	CGGAACGCCT	840
TCTGATTTAG	AGTCCTTTGC	AAAAAAACAA	AAATATATTG	AAATTATGAC	AACACATTGT	900
TTAGATTTTG	CAAGATTGTT	TCCTACTTTT	GATCCAGATC	TTTATCCAAC	AGGATCAGGT	960
GATATAAGTT	TACAAAAAAC	ACGTAGAATT	CTTCTCCTT	TTATCCCTAT	ACGTACTGCA	1020
GATGGGTTAA	CATTAAATAA	TACTTCAATT	GATACTTCAA	ATTGGCCTAA	TTATGAAAAAT	1080
GGGAATGGCG	CGTTTCCAAA	CCCAAAAGAA	AGAATATTAA	AACAATTCAA	ACTGTATCCT	1140
AGTTGGAGAG	CGGCACAGTA	CGGTGGGCTT	TTACAACCTT	ATTTATGGGC	AATAGAAGTC	1200
CAAGATTCTG	TAGAGACTCG	TTTGTATGGG	CAGCTTCCAG	CTGTAGATCC	ACAGGCAGGG	1260
CCTAATTATG	TTTCCATAGA	TTCTTCTAAT	CCAATCATAC	AAATAAATAT	GGATACTTGG	1320
AAAACACCAC	CACAAGGTGC	GAGTGGGTGG	AATACAAATT	TAATGAGAGG	AAGTGTAAGC	1380
GGGTAAAGTT	TTTTACAACG	AGATGGTACG	AGACTTAGTG	CTGGTATGGG	TGGTGGTTTT	1440
GCTGATACAA	TATATAGTCT	CCCTGCAACT	CATTATCTTT	CTTATCTCTA	TGGAACCTCT	1500
TATCAAACCT	CTGATAACTA	TTCTGGTCAC	GTTGGTGCAT	TGGTAGGTGT	GAGTACGCCT	1560
CAAGAGGCTA	CTCTTCCTAA	TATTATAGGT	CAACCAGATG	AACAGGGAAA	TGTATCTACA	1620
ATGGGATTTG	CGTTTGAAAA	AGCTTCTTAT	GGAGGTACAG	TTGTTAAAGA	ATGGTTAAAT	1680
GGTGCGAATG	CGATGAAGCT	TTCTCCTGGG	CAATCTATAG	GTATTCCTAT	TACAAATGTA	1740
ACAAGTGGAG	AATATCAAAT	TCGTTGTCGT	TATGCAAGTA	ATGATAATAC	TAACGTTTTT	1800
TTTAATGTAG	ATACTGGTGG	AGCAAATCCA	ATTTTCCAAC	AGATAAACTT	TGCATCTACT	1860
GTAGMTAATA	ATACGGGAGT	ACAAGGAGCA	AATGGTGTCT	ATGTAGTCAA	ATCTATTGCT	1920
ACAACCTGATA	ATTCTTTTAC	AGTAAAAATT	CCTGCGAAGA	CGATTAAATG	TCATTTAACC	1980
AACCAAGGTT	CTTCTGATGT	CTTTTATAGAT	CGTATTGAGT	TTGTTCCAAT	TCTAGAAATCA	2040
AATACTGTAA	CTATATTCAA	CAATTCATAT	ACTACAGGTT	CAGCAAATCT	TATACCAGCA	2100
ATAGCTCCTC	TTTGGAGTAC	TAGTTCAGAT	AAAGCCCTTA	CAGGTTCTAT	GTCAATAACA	2160
GGTCGAACCTA	CCCCTAACAG	TGATGATGCT	TTGCTTCGAT	TTTTTAAAAAC	TAATTATGAT	2220
ACACAAACCA	TTCTATTCC	GGGTTCCGGA	AAAGATTTTA	CAAATACTCT	AGAAATACAA	2280
GACATAGTTT	CTATTGATAT	TTTTGTGCGA	TCTGGTCTAC	ATGGATCCGA	TGGATCTATA	2340
AAATTAGATT	TTACCAATAA	TAATAGTGGT	AGTGGTGGCT	CTCCAAAGAG	TTTCACCGAG	2400
CALP.TGATT	TAGAGAATAT	CACAACACAA	GTGAATGCTC	TATTCACATC	TAATACACAA	2460
GATGCACTTG	CAACAGATGT	GAGTGATCAT	GATATTGAAG	AAGTGGTTCT	AAAAGTAGAT	2520
GCATTATCTG	ATGAAGTGTT	TGGAAAAGAG	AAAAAAACAT	TGCGTAAATT	TGTAAATCAA	2580
GCGAAGCGCT	TAAGCAAGGC	GCGTAATCTC	CTGGTAGGAG	GCAATTTTGA	TAACCTTGGAT	2640
GCTTGGTATA	GAGGAAGAAA	TGTAGTAAC	GTATCTAATC	ACGAAGTGT	GAAGAGTGAT	2700
CATGTATTAT	TACCACCACC	AGGATTGTCT	CCATCTTATA	TTTTCCAAAA	AGTGGAGGAA	2760

TCTAAATTAA AACGAAATAC ACGTTATACG GTTCTCGGAT TTATTGCGCA TGCAACAGAT	2820
TTAGAAATTG TGGTTTCTCG TTATGGGCAA GAAATAAAGA AAGTGGTGCA AGTTCCTTAT	2880
GGAGAAGCAT TCCCATTAAC ATCAAGTGGA CCAGTTTGTT GTATCCCACA TTCTACAAGT	2940
AATGGAACTT TAGGCAATCC ACATTTCTTT AGTTACAGTA TTGATGTAGG TGCATTAGAT	3000
GTAGACACAA ACCCTGGTAT TGAATTCGGT CTTCGTATTG TAAATCCAAC TGGAAATGGCA	3060
CGCGTAAGCA ATTTGGAAAT TCGTGAAGAT CGTCCATTAG CAGCAAATGA AATACGACAA	3120
GTACAACGTG TCGCAAGAAA TTGGAGAACC GAGTATGAGA AAGAACGTGC GGAAGTAACA	3180
AGTTTAATTC AACCTGTTAT CAATCGAATC AATGGATTGT ATGACAATGG AAATTGGAAC	3240
GGTTCATTTC GTTCAGATAT TTCGTATCAG AATATAGACG CGATTGTATT ACCAACGTTA	3300
CCAAAGTTAC GCCATTGGTT TATGTCAGAT AGATTTAGTG AACAGGAGA TATCATGGCT	3360
AAATCCAAG GTGCATTAAA TCGTGCGTAT GCACAACCTGG AACAAAATAC GCTTCTGCAT	3420
AATGGTCATT TTACAAAGA TGCAGCCAAT TGGACGGTAG AAGGCGATGC ACATCAGGTA	3480
GTATTAGAAG ATGGTAAACG TGTATTACGA TTGCCAGATT GGTCTTCGAG TGTGTCTCAA	3540
ACGATTGAAA TCGAGAATTT TGATCCAGAT AAAGAATATC AATTAGTATT TCATGGGCCAA	3600
GGAGAAGGAA CGGTTACGTT GGAGCATGGA GAAGAAACAA AATATATAGA AACGCATACA	3660
CATCATTTTG CGAATTTTAC AACTTCTCAA CGTCAAGGAC TCACGTTTGA ATCAAATAAA	3720
GTGACAGTGA CCATTTCTTC AGAAGATGGA GAATTCCTAG TGGATAATAT TCGCGCTGTG	3780
GAAGCTCCTC TTCCTACAGA TGACCAAAAT TCTGAGGGAA ATACGGCTTC CAGTACGAAT	3840
ACCGATACAA GTATGAACAA CAATCAA	3867

(2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1289 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: BACILLUS THURINGIENSIS
- (C) INDIVIDUAL ISOLATE: PS17

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC 1628) NRRL B-18652

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met	Ala	Ile	Leu	Asn	Glu	Leu	Tyr	Pro	Ser	Val	Pro	Tyr	Asn	Val	Leu
1				5					10				15		
Ala	Tyr	Thr	Pro	Pro	Ser	Phe	Leu	Pro	Asp	Ala	Gly	Thr	Gln	Ala	Thr
			20					25					30		
Pro	Ala	Asp	Leu	Thr	Ala	Tyr	Glu	Gln	Leu	Leu	Lys	Asn	Leu	Glu	Lys
			35				40					45			
Gly	Ile	Asn	Ala	Gly	Thr	Tyr	Ser	Lys	Ala	Ile	Ala	Asp	Val	Leu	Lys
			50			55					60				
Gly	Ile	Phe	Ile	Asp	Asp	Thr	Ile	Asn	Tyr	Gln	Thr	Tyr	Val	Asn	Ile
			65		70					75				80	
Gly	Leu	Ser	Leu	Ile	Thr	Leu	Ala	Val	Pro	Glu	Ile	Gly	Ile	Phe	Thr
			85					90						95	
Pro	Phe	Il	Gly	Leu	Phe	Phe	Ala	Ala	Leu	Asn	Lys	His	Asp	Ala	Pro
			100				105						110		
Pro	Pro	Pro	Asn	Ala	Lys	Asp	Ile	Phe	Glu	Ala	Met	Lys	Pro	Ala	Ile
			115				120					125			

Gln Glu Met Ile Asp Arg Thr Leu Thr Ala Asp Glu Gln Thr Phe Leu
 130 135 140
 Asn Gly Glu Ile Ser Gly Leu Gln Asn Leu Ala Ala Arg Tyr Gln Ser
 145 150 155 160
 Thr Met Asp Asp Ile Gln Ser His Gly Gly Phe Asn Lys Val Asp Ser
 165 170
 Gly Leu Ile Lys Phe Thr Asp Glu Val Leu Ser Leu Asn Ser Phe
 180 185 190
 Tyr Thr Asp Arg Leu Pro Val Phe Ile Thr Asp Asn Thr Ala Asp Arg
 195 200 205
 Thr Leu Leu Gly Leu Pro Tyr Tyr Ala Ile Leu Ala Ser Met His Leu
 210 215 220
 Met Leu Leu Arg Asp Ile Ile Thr Lys Gly Pro Thr Trp Asp Ser Lys
 225 230 235 240
 Ile Asn Phe Thr Pro Asp Ala Ile Asp Ser Phe Lys Thr Asp Ile Lys
 245 250 255
 Asn Asn Ile Lys Leu Tyr Ser Lys Thr Ile Tyr Asp Val Phe Gln Lys
 260 265 270
 Gly Leu Ala Ser Tyr Gly Thr Pro Ser Asp Leu Glu Ser Phe Ala Lys
 275 280 285
 Lys Gln Lys Tyr Ile Glu Ile Met Thr Thr His Cys Leu Asp Phe Ala
 290 295 300
 Arg Leu Phe Pro Thr Phe Asp Pro Asp Leu Tyr Pro Thr Gly Ser Gly
 305 310 315 320
 Asp Ile Ser Leu Gln Lys Thr Arg Arg Ile Leu Ser Pro Phe Ile Pro
 325 330 335
 Ile Arg Thr Ala Asp Gly Leu Thr Leu Asn Asn Thr Ser Ile Asp Thr
 340 345 350
 Ser Asn Trp Pro Asn Tyr Glu Asn Gly Asn Gly Ala Phe Pro Asn Pro
 355 360 365
 Lys Glu Arg Ile Leu Lys Gln Phe Lys Leu Tyr Pro Ser Trp Arg Ala
 370 375 380
 Ala Gln Tyr Gly Gly Leu Leu Gln Pro Tyr Leu Trp Ala Ile Glu Val
 385 390 395 400
 Gln Asp Ser Val Glu Thr Arg Leu Tyr Gly Gln Leu Pro Ala Val Asp
 405 410 415
 Pro Gln Ala Gly Pro Asn Tyr Val Ser Ile Asp Ser Ser Asn Pro Ile
 420 425 430
 Ile Gln Ile Asn Met Asp Thr Trp Lys Thr Pro Pro Gln Gly Ala Ser
 435 440 445
 Gly Trp Asn Thr Asn Leu Met Arg Gly Ser Val Ser Gly Leu Ser Phe
 450 455 460
 Leu Gln Arg Asp Gly Thr Arg Leu Ser Ala Gly Met Gly Gly Gly Phe
 465 470 475 480
 Ala Asp Thr Ile Tyr Ser Leu Pro Ala Thr His Tyr Leu Ser Tyr Leu
 485 490 495
 Tyr Gly Thr Pro Tyr Gln Thr Ser Asp Asn Tyr Ser Gly His Val Gly
 500 505 510
 Ala Leu Val Gly Val Ser Thr Pro Gln Glu Ala Thr Leu Pro Asn Ile
 515 520 525
 Ile Gly Gln Pro Asp Glu Gln Gly Asn Val Ser Thr Met Gly Phe Pro
 530 535 540
 Phe Glu Lys Ala Ser Tyr Gly Gly Thr Val Val Lys Glu Trp Leu Asn
 545 550 555 560
 Gly Ala Asn Ala Met Lys Leu Ser Pro Gly Gln Ser Ile Gly Ile Pro
 565 570 575
 Ile Thr Asn Val Thr Ser Gly Glu Tyr Gln Ile Arg Cys Arg Tyr Ala
 580 585 590

Ser Asn Asp Asn Thr Asn Val Phe Phe Asn Val Asp Thr Gly ly Ala
 595 600
 Asn Pro Ile Phe Gln Gln Ile Asn Phe Ala Ser Thr Val Asp Asn Asn
 610 615 620
 Thr Gly Val Gln Gly Ala Asn Gly Val Tyr Val Val Lys Ser Ile Ala
 625 630 635 640
 Thr Thr Asp Asn Ser Phe Thr Val Lys Ile Pro Ala Lys Thr Ile Asn
 645 650 655
 Val His Leu Thr Asn Gln Gly Ser Ser Asp Val Phe Leu Asp Arg Ile
 660 665 670
 Glu Phe Val Pro Ile Leu Glu Ser Asn Thr Val Thr Ile Phe Asn Asn
 675 680 685
 Ser Tyr Thr Thr Gly Ser Ala Asn Leu Ile Pro Ala Ile Ala Pro Leu
 690 695 700
 Trp Ser Thr Ser Ser Asp Lys Ala Leu Thr Gly Ser Met Ser Ile Thr
 705 710 715 720
 Gly Arg Thr Thr Pro Asn Ser Asp Asp Ala Leu Leu Arg Phe Phe Lys
 725 730 735
 Thr Asn Tyr Asp Thr Gln Thr Ile Pro Ile Pro Gly Ser Gly Lys Asp
 740 745 750
 Phe Thr Asn Thr Leu Glu Ile Gln Asp Ile Val Ser Ile Asp Ile Phe
 755 760 765
 Val Gly Ser Gly Leu His Gly Ser Asp Gly Ser Ile Lys Leu Asp Phe
 770 775 780
 Thr Asn Asn Asn Ser Gly Ser Gly Gly Ser Pro Lys Ser Phe Thr Glu
 785 790 795 800
 Gln Asn Asp Leu Glu Asn Ile Thr Thr Gln Val Asn Ala Leu Phe Thr
 805 810 815
 Ser Asn Thr Gln Asp Ala Leu Ala Thr Asp Val Ser Asp His Asp Ile
 820 825 830
 Glu Glu Val Val Leu Lys Val Asp Ala Leu Ser Asp Glu Val Phe Gly
 835 840 845
 Lys Glu Lys Lys Thr Leu Arg Lys Phe Val Asn Gln Ala Lys Arg Leu
 850 855 860
 Ser Lys Ala Arg Asn Leu Leu Val Gly Gly Asn Phe Asp Asn Leu Asp
 865 870 875 880
 Ala Trp Tyr Arg Gly Arg Asn Val Val Asn Val Ser Asn His Glu Leu
 885 890 895
 Leu Lys Ser Asp His Val Leu Leu Pro Pro Pro Gly Leu Ser Pro Ser
 900 905 910
 Tyr Ile Phe Gln Lys Val Glu Glu Ser Lys Leu Lys Arg Asn Thr Arg
 915 920 925
 Tyr Thr Val Ser Gly Phe Ile Ala His Ala Thr Asp Leu Glu Ile Val
 930 935 940
 Val Ser Arg Tyr Gly Gln Glu Ile Lys Lys Val Val Gln Val Pro Tyr
 945 950 955 960
 Gly Glu Ala Phe Pro Leu Thr Ser Ser Gly Pro Val Cys Cys Ile Pro
 965 970 975
 His Ser Thr Ser Asn Gly Thr Leu Gly Asn Pro His Phe Phe Ser Tyr
 980 985 990
 Ser Ile Asp Val Gly Ala Leu Asp Val Asp Thr Asn Pro Gly Ile Glu
 995 1000 1005
 Phe Gly Leu Arg Ile Val Asn Pro Thr Gly Met Ala Arg Val Ser Asn
 1010 1015 1020
 Leu Glu Ile Arg lu Asp Arg Pro Leu Ala Ala Asn Glu Ile Arg Gln
 1025 1030 1035 1040
 Val Gln Arg Val Ala Arg Asn Trp Arg Thr Glu Tyr Glu Lys Glu Arg
 1045 1050 1055

35

Ala Glu Val Thr Ser Leu Ile Gln Pro Val Ile Asn Arg Ile Asn ly
 1060 1065 1070
 Leu Tyr Asp Asn Gly Asn Trp Asn Gly Ser Ile Arg Ser Asp Ile Ser
 1075 1080 1085
 Tyr Gln Asn Ile Asp Ala Ile Val Leu Pro Thr Leu Pro Lys Leu Arg
 1090 1095 1100
 His Trp Phe Met Ser Asp Arg Phe Ser Glu Gln Gly Asp Ile Met Ala
 1105 1110 1115 1120
 Lys Phe Gln Gly Ala Leu Asn Arg Ala Tyr Ala Gln Leu Glu Gln Asn
 1125 1130 1135
 Thr Leu Leu His Asn Gly His Phe Thr Lys Asp Ala Ala Asn Trp Thr
 1140 1145 1150
 Val Glu Gly Asp Ala His Gln Val Val Leu Glu Asp Gly Lys Arg Val
 1155 1160 1165
 Leu Arg Leu Pro Asp Trp Ser Ser Ser Val Ser Gln Thr Ile Glu Ile
 1170 1175 1180
 Glu Asn Phe Asp Pro Asp Lys Glu Tyr Gln Leu Val Phe His Gly Gln
 1185 1190 1195 1200
 Gly Glu Gly Thr Val Thr Leu Glu His Gly Glu Glu Thr Lys Tyr Ile
 1205 1210 1215
 Glu Thr His Thr His His Phe Ala Asn Phe Thr Thr Ser Gln Arg Gln
 1220 1225 1230
 Gly Leu Thr Phe Glu Ser Asn Lys Val Thr Val Thr Ile Ser Ser Glu
 1235 1240 1245
 Asp Gly Glu Phe Leu Val Asp Asn Ile Ala Leu Val Glu Ala Pro Leu
 1250 1255 1260
 Pro Thr Asp Asp Gln Asn Ser Glu Gly Asn Thr Ala Ser Ser Thr Asn
 1265 1270 1275 1280
 Ser Asp Thr Ser Met Asn Asn Asn Gln
 1285

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3771 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Bacillus thuringiensis*
- (C) INDIVIDUAL ISOLATE: 33f2

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC 2316) B-18785

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 4..24
- (D) OTHER INFORMATION: /function= "oligonucleotide
 hybridization probe"
 /product= "GCA/T ACA/T TTA AAT GAA GTA/T TAT"
 /standard name= "probe a"
 /note= "Probe A"

(ix) FEATURE:

- (A) NAME/KEY: misc feature
- (B) LOCATION: 13..33
- (D) OTHER INFORMATION: /function= "oligonucleotide
 hybridization probe"
 /product= "AAT GAA GTA/T TAT CCA/T GTA/T AAT"
 /standard name= "Probe B"
 /lab id= pProbe-b
 /note= "probe b"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATGGCTACAC	TTAATGAAGT	ATATCCTGTG	AATTATAATG	TATTATCTTC	TGATGCTTTT	60
CAACAATTAG	ATACAACAGG	TTTTAAAAGT	AAATATGATG	AAATGATAAA	AGCATTCGAA	120
AAAAAATGGA	AAAAAGGGGC	AAAAGGAAAA	GACCTTTTAG	ATGTTGCATG	GACTTATATA	180
ACTACAGGAG	AAATTGACCC	TTTAAATGTA	ATTAAAGGTG	TTTATCTGTATT	AACCTTTA	240
ATTCTGTAAG	TTGGTACTGT	GGCCTCTGCA	GCAAGTACTA	TTGTAAGTTT	TATTTGGCCT	300
AAAAATATTTG	GAGATAAAC	AAATGCABAA	AATATATTTG	AAGAGCTCAA	GCCTCAAATT	360
GAAGCATTAA	TTCAACAAGA	TATAACAAAC	TATCAAGATG	CAATTAATCA	AAAAAAATTT	420
GACAGTCTTC	AGAAAACAAT	TAATCTATAT	ACAGTAGCTA	TAGATAACAA	TGATTACGTA	480
ACAGCAAAAA	CGCAACTCGA	AAATCTAAAT	TCTATACTTA	CCTCAGATAT	CTCCATATTT	540
ATTCCAGAAG	GATATGAAAC	TGGAGGTTTA	CCTTATTATG	CTATGGTTGC	TAATGCTCAT	600
ATATTATTGT	TAAGAGACGC	TATAGTTAAT	GCAGAGAAAT	TAGGCTTTAG	TGATAAAGAA	660
GTAGACACAC	ATAAAAAATA	TATCAAAATG	ACAATACACA	ATCATACTGA	AGCAGTAATA	720
AAAGCATTCT	TAAATGGACT	TGACAAATTT	AAGAGTTTAG	ATGTAAATAG	CTATAATAAA	780
AAAGCAAATT	ATATTAAAGG	TATGACAGAA	ATGGTTCTTG	ATCTAGTTGC	TCTATGCCCA	840
ACTTTGATC	CAGATCATT	TCAAAAAGAA	GTAGAAATTG	AATTTACAAG	AACTATTTCT	900
TCTCCAATTT	ACCAACCTGT	ACCTAAAAAC	ATGCAAAATA	CCTCTAGCTC	TATTGTACCT	960
AGCGATCTAT	TTCACTATCA	AGGAGATCTT	GTAAAATTAG	AATTTTCTAC	AAGAACGGAC	1020
AACGATGGTC	TTGCAAAAAT	TTTTACTGGT	ATTGCAAAAC	CATTCTACAA	ATGCGCTAAT	1080
ACTCATGAAA	CATACCATGT	AGATTTTAGT	TATAATACCC	AATCTAGTGG	TAATATTTCA	1140
AGAGGCTCTT	CAAATCCGAT	TCCAATTGAT	CCTAATAATC	CCATTATTTT	AACTTGTATT	1200
AGAAATTCAT	TTTATAAGGC	AATAGCGGGA	TCTTCTGTTT	TAGTTAATTT	TAAAGATGGC	1260
ACTCAAGGGT	ATGCATTTGC	CCAAGCACCA	ACAGGAGGTG	CCTGGGACCA	TTCTTTTATT	1320
GAATCTGATG	GTGCCCCAGA	AGGGCATAAA	TTAAACTATA	TTTATACTTC	TCCAGGTGAT	1380
ACATTAAGAG	ATTTTCATCA	TGTATATACT	CTTATAAGTA	CTCCAACTAT	AAATGAACTA	1440
TCAACAGAAA	AAATCAAAGG	CTTTCCTGCG	GAAAAAGGAT	ATATCAAAAA	TCAAGGGATC	1500
ATGAAATATT	ACGGTAAACC	AGAATATATT	AATGGAGCTC	AACCAGTTAA	TCTGGAAAAC	1560
CAGCAAAACAT	TAATATTGGA	ATTTTCATGCT	TCAAAAACAG	CTCAATATAC	CATTGCTATA	1620
CGTTATGCCA	GTACCCCAAG	AACAAAAGGT	TATTTTCGTT	TAGATAATCA	GGAAGTGCAC	1680
ACGCTTAATA	TACCTACTTC	ACACAACGGT	TATGTAACCG	GTAATATTGG	TGAAAATTAT	1740
GATTTATATA	CAATAGGTTT	ATATACAATT	ACAGAAGGTA	ACCATACTCT	TCBAATCCAA	1800
CATAATGATA	AAAATGGAAT	GGTTTATGAT	CGTATTGAAT	TTGTTCCCTA	AGATTCACCT	1860
CAAGATTCAC	CTCAAGATTC	ACCTCCAGAA	GTTCCAGGAT	CAACAATTAT	TTTTGATAAA	1920
TCATCTCCAA	CTATATGGTC	TTCTAACAAA	CATCTATATA	GCCATATACA	TTTGAAGGGA	1980
TCATATACAA	GTCAGGGAAG	TTATCCACAC	AATTTATTAA	TTAATTTATT	TCATCCTACA	2040
GACCCTAACA	GAAATCATA	TATTCATGTT	AACAATGGTG	ATATGAATGT	TGATTATGGA	2100
AAAGATTCTG	TAGCCGATGG	GTTAAATTTT	AATAAAATAA	CTGCTACGAT	ACCAAGTGAT	2160
GCTTGGTATA	GCGGTACTAT	TACTTCTATG	CACCTATTTA	ATGATAATAA	TTTTAAACAA	2220
ATAACTCCTA	AATTTGAACT	TTCTAATGAA	TTAGAAAACA	TCACAACTCA	AGTAAATGCT	2280
TTATTGCGAT	CTAGTGCACA	AGATACTCTC	GCAAGTAATG	TAAGTGATTA	CTGGATTGAA	2340
CAGGTCGTTA	TGAAAGTCGA	TGCCCTTATCA	GATGAAGTAT	TTGGAAAAGA	GAAAAAAGCA	2400
TTACGTAAAT	TGGTAAATCA	AGCAAAACGT	CTCAGTAAAA	TACGAAATCT	TCTCATAGGT	2460
GGTAATTTTG	ACAATTTAGT	CGCTTGGTAT	ATGGGAAAAG	ATGTAGTAAA	AGAATCGGAT	2520
CATGAATTAT	TTAAAAGTGA	TCATGTCTTA	CTACCTCCCC	CAACATTCCA	TCCTTCTTAT	2580

ATTTTCCAAA	AGGTGGAAGA	ATCAAACTA	AAACCAAATA	CACGTTATAC	TATTTCTGGT	2640
TTTATCGCAC	ATGGAGAAGA	TGTAGAGCTT	TTGTCTCTC	GTTATGGGCA	AGAAATACAA	2700
AAAGTGAATG	AAGTGCCATA	TGAAGAAGCA	CTTCCTCTTA	CATCTGAATC	TAATTCTAGT	2760
TGTTGTGTTT	CAAATTTAAA	TATAAATGAA	ACACTAGCTG	ATCCACATTT	CTTTAGTTAT	2820
AGCATCGATG	TGGTTCTCT	GGAAATGGAA	GCGAATCCTG	GTATTGAATT	TGGTCTCCGT	2880
ATTGTCAAAC	CAACAGGTAT	GGCACGTGTA	AGTAATTTAG	AAATTCGAGA	AGACCGTCCA	2940
TTAACAGCAA	AAGAAATTCG	TCAAGTACAA	CGTGACGCAA	GAGATTGGAA	ACAAAACATAT	3000
GAACAAGAAC	GAACAGAGAT	CACAGCTATA	ATTCAACCTG	TTCTTAATCA	AATTAATGCG	3060
TTATACGAAA	ATGAAGATTG	GAATGTTCT	ATTCGTTCAA	ATGTTTCCTA	TCATGATCTA	3120
GAGCAAATTA	TGCTTCCTAC	TTTATTAAAA	ACTGAGGAAA	TAAATTGTAA	TTATGATCAT	3180
CCAGCTTTTT	TATTAAAAGT	ATATCATTTG	TTTATGACAG	ATCGTATAGG	AGAACATGGT	3240
ACTATTTTAG	CACGTTTCCA	AGAAGCATT	GATCGTGCAT	ATACACAATT	AGAAAGTCGT	3300
AATCTCCTGC	ATAACGGTCA	TTTTACAAC	GATACAGCGA	ATTGGACAAT	AGAAGGAGAT	3360
GCCCATCATA	CAATCTTAGA	AGATGGTAGA	CGTGTGTTAC	GTTTACCAGA	TTGGTCTTCT	3420
AATGCAACTC	AAACAATTGA	AATTGAAGAT	TTTGACTTAG	ATCAAGAATA	CCAATTGCTC	3480
ATTCATGCAA	AAGGAAAAGG	TTCCATTACT	TTACAACATG	GAGAGAAAAA	CGAATATGTG	3540
GAAACACATA	CTCATCATAC	AAATGATTTT	ATAACATCCC	AAAATATTCC	TTTCACTTTT	3600
AAAGGAAATC	AAATTGAAGT	CCATATTACT	TCAGAAGATG	GAGAGTTTTT	AATCGATCAC	3660
ATTACAGTAA	TAGAAGTTTC	TAAAACAGAC	ACAAATACAA	ATATTATTGA	AAATTCACCA	3720
ATCAATACAA	GTATGAATAG	TAATGTAAGA	GATAGATATAC	CAAGAAGTCT	C	3771

(2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1425 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: BACILLUS THURINGIENSIS
 - (C) INDIVIDUAL ISOLATE: PS52A1
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC 2321) B-18770
- (ix) FEATURE:
 - (A) NAME/KEY: mat peptide
 - (B) LOCATION: 1..1425
 - (D) OTHER INFORMATION: /product= "OPEN READING FRAME OF MATURE PROTEIN"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

ATGATTATTG	ATAGTAAAAC	GACTTTACCT	AGACATTAC	TTATTCATAC	AATTAAATTA	60
AATTCTAATA	AGAAATATGG	TCCTGGTGAT	ATGACTAATG	GAAATCAATT	TATTATTTC	120
AAACAAGAAT	GGGCTACGAT	TGGAGCATAT	ATTCAGACTG	GATTAGGTTT	ACCGTAAAT	180
GAACAACAAT	TAAGAACACA	TGTTAATTTA	AGTCAGGATA	TATCAATACC	TAGTGATTTT	240
TCTCAATTAT	ATGATGTTTA	TTGTTCTGAT	AAAACCTCAG	CAGAAATGGT	GAATAAAAAT	300
TTATATCCTT	TAATTATTAA	ATCTGCTAAT	GATATTGCTT	CATATGGTTT	TAAAGTTGCT	360
GGTGATCCTT	CTATTAAAGAA	AGATGGATAT	TTTAAAAAAT	TGCAAGATGA	ATTAGATAAT	420
ATTGTTGATA	ATAATCCGA	TGATGATGCA	ATAGCTAAAG	CTATTAAAGA	TTTTAAAGCG	480
CGATGTGGTA	TTTTAATTAA	AGAAGCTAAA	CAATATGAAG	AAGCTGCAAA	AAATATTGTA	540

ACATCTTTAG	ATCAATTTTT	ACATGGTGAT	CAGAAAAAAT	TAGAAGGTGT	TATCAATATT	600
CAAAAACGTT	TAAAAGAACT	TCAAACAGCT	CTTAATCAAG	CCCATGGGGA	AAGTAGTCCA	660
GCTCATAAAG	AGTTATTAGA	AAAAGTAAAA	AATTTAAAAA	CAACATTAGA	AAGGACTATT	720
AAAGCTGAAC	AAGATTTAGA	GAAAAAAGTA	GAATATAGTT	TTCTATTAGG	ACCATTTGTTA	780
GGATTTGTTG	TTTATGAAAT	TCTTGAAAAT	ACTGCTGTTC	AGCATATAAA	AAATCAAATT	840
GATGAGATAA	AGAAACAATT	AGATTCTGCT	CAGCATGATT	TGGATAGAGA	TGTTAAAATT	900
ATAGGAATGT	TAAATAGTAT	TAATACAGAT	ATTGATAATT	TATATAGTCA	AGGACAAGAA	960
GCAATTAAAG	TTTTCCAAAA	GTTACAAGGT	ATTTGGGCTA	CTATTGGAGC	TCAAATAGAA	1020
AATCTTAGAA	CAACGTCGTT	ACAAGAAGTT	CAAGATTCTG	ATGATGCTGA	TGAGATACAA	1080
ATTGAACTTG	AGGACGCTTC	TGATGCTTGG	TTAGTTGTGG	CTCAAGAAGC	TCGTGATTTT	1140
ACACTAAATG	CTTATTCAAC	TAATAGTAGA	CAAAATTTAC	CGATTAAATG	TATATCAGAT	1200
TCATGTAATT	GTTCAACAAC	AAATATGACA	TCAAATCAAT	ACAGTAATCC	AACAACAAAT	1260
ATGACATCAA	ATCAATATAT	GATTTACAT	GAATATACAA	GTTTACCAAA	TAATTTTATG	1320
TTATCAAGAA	ATAGTAATTT	AGAATATAAA	TGTCTGAAA	ATAATTTTAT	GATATATTGG	1380
TATAATAATT	CGGATTGGTA	TAATAATTGG	GATTGGTATA	ATAAT		1425

(2) INFORMATION FOR SEQ ID NO:7 (PS52A1):

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 475 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: BACILLUS THURINGIENSIS
 - (C) INDIVIDUAL ISOLATE: PS52A1
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522 (pMYC 2321) B-18770
- (ix) FEATURE:
 - (A) NAME/KEY: Protein
 - (B) LOCATION: 1..475

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Met	Ile	Ile	Asp	Ser	Lys	Thr	Thr	Leu	Pro	Arg	His	Ser	Leu	Ile	His	1	5	10	15
Thr	Ile	Lys	Leu	Asn	Ser	Asn	Lys	Lys	Tyr	Gly	Pro	Gly	Asp	Met	Thr	20	25	30	
Asn	Gly	Asn	Gln	Phe	Ile	Ile	Ser	Lys	Gln	Glu	Trp	Ala	Thr	Ile	Gly	35	40	45	
Ala	Tyr	Ile	Gln	Thr	Gly	Leu	Gly	Leu	Pro	Val	Asn	Glu	Gln	Gln	Leu	50	55	60	
Arg	Thr	His	Val	Asn	Leu	Ser	Gln	Asp	Ile	Ser	Ile	Pro	Ser	Asp	Phe	65	70	75	80
Ser	Gln	Leu	Tyr	Asp	Val	Tyr	Cys	Ser	Asp	Lys	Thr	Ser	Ala	Glu	Trp	85	90	95	
Trp	Asn	Lys	Asn	Leu	Tyr	Pro	Leu	Ile	Ile	Lys	Ser	Ala	Asn	Asp	Ile	100	105	110	
Ala	Ser	Tyr	Gly	Phe	Lys	Val	Ala	Gly	Asp	Pro	Ser	Ile	Lys	Lys	Asp	115	120	125	
Gly	Tyr	Ph	Lys	Lys	Leu	Gln	Asp	lu	Leu	Asp	Asn	Ile	Val	Asp	Asn	130	135	140	
Asn	Ser	Asp	Asp	Asp	Ala	Ile	Ala	Lys	Ala	Ile	Lys	Asp	Phe	Lys	Ala	145	150	155	160

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Arg Cys ly Ile Leu Ile Lys Glu Ala Lys In Tyr lu Glu Ala Ala
 165 170 175
 Lys Asn Ile Val Thr Ser Leu Asp Gln Phe Leu His Gly Asp Gln Lys
 180 185 190
 Lys Leu Glu Gly Val Ile Asn Ile Gln Lys Arg Leu Lys Glu Val Gln
 195 200 205
 Thr Ala Leu Asn Gln Ala His Gly Glu Ser Ser Pro Ala His Lys Glu
 210 215 220
 Leu Leu Glu Lys Val Lys Asn Leu Lys Thr Thr Leu Glu Arg Thr Ile
 225 230 235 240
 Lys Ala Glu Gln Asp Leu Glu Lys Lys Val Glu Tyr Ser Phe Leu Leu
 245 250 255
 Gly Pro Leu Leu Gly Phe Val Val Tyr Glu Ile Leu Glu Asn Thr Ala
 260 265 270
 Val Gln His Ile Lys Asn Gln Ile Asp Glu Ile Lys Lys Gln Leu Asp
 275 280 285
 Ser Ala Gln His Asp Leu Asp Arg Asp Val Lys Ile Ile Gly Met Leu
 290 295 300
 Asn Ser Ile Asn Thr Asp Ile Asp Asn Leu Tyr Ser Gln Gly Gln Glu
 305 310 315 320
 Ala Ile Lys Val Phe Gln Lys Leu Gln Gly Ile Trp Ala Thr Ile Gly
 325 330 335
 Ala Gln Ile Glu Asn Leu Arg Thr Thr Ser Leu Gln Glu Val Gln Asp
 340 345 350
 Ser Asp Asp Ala Asp Glu Ile Gln Ile Glu Leu Glu Asp Ala Ser Asp
 355 360 365
 Ala Trp Leu Val Val Ala Gln Glu Ala Arg Asp Phe Thr Leu Asn Ala
 370 375 380
 Tyr Ser Thr Asn Ser Arg Gln Asn Leu Pro Ile Asn Val Ile Ser Asp
 385 390 395 400
 Ser Cys Asn Cys Ser Thr Thr Asn Met Thr Ser Asn Gln Tyr Ser Asn
 405 410 415
 Pro Thr Thr Asn Met Thr Ser Asn Gln Tyr Met Ile Ser His Glu Tyr
 420 425 430
 Thr Ser Leu Pro Asn Asn Phe Met Leu Ser Arg Asn Ser Asn Leu Glu
 435 440 445
 Tyr Lys Cys Pro Glu Asn Asn Phe Met Ile Tyr Trp Tyr Asn Asn Ser
 450 455 460
 Asp Trp Tyr Asn Asn Ser Asp Trp Tyr Asn Asn
 465 470 475

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - {A} LENGTH: 1185 base pairs
 - {B} TYPE: nucleic acid
 - {C} STRANDEDNESS: double
 - {D} TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - {A} ORGANISM: BACILLUS THURINGIENSIS
 - {C} INDIVIDUAL ISOLATE: PS69D1
- (vii) IMMEDIATE SOURCE:
 - {B} CLONE: E. coli NM522(pMYC2317) NRRL B-18816
- (ix) FEATURE:
 - {A} NAME/KEY: mat peptide
 - {B} LOCATION: 1..1185

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGATTTTAG	GGAATGGAAA	GACTTTACCA	AAGCATATAA	GATTAGCTCA	TATTTTTCGA	60
ACACAGAATT	CTTCAGCTAA	GAAAGACAAT	CCTCTTGGAC	CAGAGGGGAT	GGTTACTAAA	120
GACGGTTTTA	TAATCTCTAA	GGAAGAATGG	GCATTGTGTC	AGGCCTATGT	GACTACAGGC	180
ACTGGTTTAC	CTATCAATGA	CGATGAGATG	CGTAGACATG	TTGGGTTACC	ATCAGGCATT	240
CAAAATCCTG	ATGATTTTAA	TCAATTATAT	AAGGTTTATA	ATGAAGATAA	ACATTTATGC	300
AGTTGGTGGA	ATGGTTTCTT	GTTTCCATTA	GTTCTTAAAA	CAGCTAATGA	TATTTCCGCT	360
TACGGATTTA	AATGTGCTGG	AAAGGGTGCC	ACTAAAGGAT	ATTATGAGGT	CATGCAAGAC	420
GATGTAGAAA	ATATTTTACA	TAATGGTTAT	GATAAAGTTG	CACAAGAAAA	AGCACATAAG	480
GATCTGCAGG	CGGTTGTAA	AATCCTTATT	AAGGAGGCTG	ATCAATATAA	AGCTGCAGCG	540
GATGATGTTT	CAAAACATTT	AAACACATTT	CTTAAAGGCG	GTCAAGATTC	AGATGGCAAT	600
GATGTTTATG	GCCTAGAGGC	TGTTCAAGTA	CAACTAGCAC	AAGTAAAAGA	TAATCTTGAT	660
GGCCTATATG	GCGACAAAAG	CCCAGACAT	GRAGAGTTAC	TAAAGAAAGT	AGACGACCTG	720
AAAAAAGAGT	TGGAAGCTGC	TATTAAGCA	GAGAATGAAT	TAGAAAAGAA	AGTGAAAATG	780
AGTTTGTCTT	TAGGACCATT	ACTTGGATTT	GTTGTATATG	AAATCTTAGA	GCTAAGTGGC	840
GTCAAAGTA	TACACAAGAA	AGTTGAGGCA	CTACAGCCG	AGCTTGACAC	TGCTAATGAT	900
GAACTCGACA	GAGATGTAAA	AATCITAGGA	ATGATGAATA	GCATTGACAC	TCATATTGAC	960
AACATGTTAG	AGCAAGGTGA	GCAAGCTCTT	GTTGTATTTA	GAAAAATTGC	AGGCATTTGG	1020
AGTGTATATA	GTCTTAATAT	CGGCAATCTT	CGAGAAACAT	CTTAAAGA	GATAGAAGAA	1080
GAAATGATG	ACGATGCACT	GTATATTGAG	CTTGGTGATG	CGGCTGGTCA	ATGGAAGAG	1140
ATAGCCGAGG	AGGCACAATC	CTTTGTACTA	AATGCTTATA	CTCCT		1185

(2) INFORMATION FOR SEQ ID NO:9:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 395 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: BACILLUS THURINGIENSIS
 (C) INDIVIDUAL ISOLATE: PS69D1
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: E. coli NM522(pMYC2317) NRRL B-18816
- (ix) FEATURE:
 (A) NAME/KEY: Protein
 (B) LOCATION: 1..395

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met	Ile	Leu	Gly	Asn	Gly	Lys	Thr	Leu	Pro	Lys	His	Ile	Arg	Leu	Ala
1				5					10					15	
His	Ile	Phe	Ala	Thr	Gln	Asn	Ser	Ser	Ala	Lys	Lys	Asp	Asn	Pro	Leu
		20					25						30		
Gly	Pro	Glu	Gly	Met	Val	Thr	Lys	Asp	Gly	Phe	Ile	Ile	Ser	Lys	Glu
		35				40					45				
Glu	Trp	Ala	Phe	Val	Gln	Ala	Tyr	Val	Thr	Thr	Gly	Thr	Gly	Leu	Pro
	50				55					60					
Ile	Asn	Asp	Asp	Glu	Met	Arg	Arg	His	Val	Gly	Leu	Pro	Ser	Arg	Ile
65					70				75					80	
Gln	Ile	Pro	Asp	Asp	Phe	Asn	Gln	Leu	Tyr	Lys	Val	Tyr	Asn	Leu	Asp
			85					90						95	

41

Lys His Leu Cys Ser Trp Trp Asn ly Phe Leu Phe Pro Leu Val Leu
 100 105 110
 Lys Thr Ala Asn Asp Ile Ser Ala Tyr Gly Phe Lys Cys Ala Gly Lys
 115 120 125
 Gly Ala Thr Lys Gly Tyr Tyr Glu Val Met Gln Asp Val Glu Asn
 130 135 140
 Ile Ser Asp Asn Gly Tyr Asp Lys Val Ala Gln Glu Lys Ala His Lys
 145 150 155 160
 Asp Leu Gln Ala Arg Cys Lys Ile Leu Ile Lys Glu Ala Asp Gln Tyr
 165 170 175
 Lys Ala Ala Ala Asp Asp Val Ser Lys His Leu Asn Thr Phe Leu Lys
 180 185 190
 Gly Gly Gln Asp Ser Asp Gly Asn Asp Val Ile Gly Val Glu Ala Val
 195 200 205
 Gln Val Gln Leu Ala Gln Val Lys Asp Asn Leu Asp Gly Leu Tyr Gly
 210 215 220
 Asp Lys Ser Pro Arg His Glu Glu Leu Leu Lys Lys Val Asp Asp Leu
 225 230 235 240
 Lys Lys Glu Leu Glu Ala Ala Ile Lys Ala Glu Asn Glu Leu Glu Lys
 245 250 255
 Lys Val Lys Met Ser Phe Ala Leu Gly Pro Leu Leu Gly Phe Val Val
 260 265 270
 Tyr Glu Ile Leu Glu Leu Thr Ala Val Lys Ser Ile His Lys Lys Val
 275 280 285
 Glu Ala Leu Gln Ala Glu Leu Asp Thr Ala Asn Asp Glu Leu Asp Arg
 290 295 300
 Asp Val Lys Ile Leu Gly Met Met Asn Ser Ile Asp Thr Asp Ile Asp
 305 310 315 320
 Asn Met Leu Glu Gln Gly Glu Gln Ala Leu Val Val Phe Arg Lys Ile
 325 330 335
 Ala Gly Ile Trp Ser Val Ile Ser Leu Asn Ile Gly Asn Leu Arg Glu
 340 345 350
 Thr Ser Leu Lys Glu Ile Glu Glu Glu Asn Asp Asp Ala Leu Tyr
 355 360 365
 Ile Glu Leu Gly Asp Ala Ala Gly Gln Trp Lys Glu Ile Ala Glu Glu
 370 375 380
 Ala Gln Ser Phe Val Leu Asn Ala Tyr Thr Pro
 385 390 395

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 bases
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AGATRKWTW AATGGWGCKM AW

22

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Pro Thr Phe Asp Pro Asp Leu Tyr
 1 5

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Ala Ile Leu Asn Glu Leu Tyr Pro Ser Val Pro Tyr Asn Val
 1 5 10

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 14 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Ala Ile Leu Asn Glu Leu Tyr Pro Ser Val Pro Tyr Asn Val
 1 5 10

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 17 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Met Ile Ile Asp Ser Lys Thr Thr Leu Pro Arg His Ser Leu Ile Asn
 1 5 10 15
 Thr

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 24 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Met Ile Leu Gly Asn Gly Lys Thr Leu Pro Lys His Ile Arg Leu Ala
 1 5 10 15
 His Ile Phe Ala Thr Gln Asn Ser
 20

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 23 bases
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GCAATTTTAA ATGAATTATA TCC

23

(2) INFORMATION FOR SEQ ID NO:17:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 56 bases
 (B) TYPE: nucleic acid

43

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

ATGATTATTG ATTCTAAAC AACATTACCA AGACATTCWT TAATWAATAC WATWAA

56

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

AAACATATTA GATTAGCACA TATTTTTCGA ACACAAAA

38

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CAATTACAAG CWCAACC

17

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AGGAACAAAY TCAAKWCGRT CTA

23

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TGGAATAAAT TCAATTYKRT CWA

23

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGATTTTWT CAATTATATR ARGTTTAT

28

(2) INFORMATION FOR SEQ ID NO:23:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

AAGAGTTAYT ARARAAAGTA

20

(2) INFORMATION FOR SEQ ID NO:24:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TTAGGACCAT TRYTWGGATT TGTGTGTAT GAAAT

35

(2) INFORMATION FOR SEQ ID NO:25:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

GAYAGAGATG TWAAAATYWT AGGAATG

27

(2) INFORMATION FOR SEQ ID NO:26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 bases
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (synthetic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTMTAAAWC WGCTAATGAT ATT

23

(2) INFORMATION FOR SEQ ID NO:27:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1425 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: BACILLUS THURINGIENSIS
(C) INDIVIDUAL ISOLATE: PS86A1

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC1638) NRRL B-18751

(ix) FEATURE:

- (A) NAME/KEY: mat peptide
(B) LOCATION: 1..1425

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:27:

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ATGATTATTG ATAGTAAAC GACTTTACCT AGACATTCAC TTATTCATAC AATTAAATTA      60
AATTCTAATA AGAAATATGG TCCTGGTGAT ATGACTAATG GAAATCAATT TATTATTTC      120
AAACAAGAAT GGGCTACGAT TGGAGCATAT ATTCAGACTG GATTAGGTTT ACCAGTAAAT      180
GAACAACAAT TAAGAACACA TGTTAATTTA AGTCAGGATA TATCAATACC TAGTGATTTT      240
TCTCAATTAT ATGATGTTTA TTGTTCTGAT AAAACTTCAG CAGAATGGTG GAATAAAAT      300
TTATATCCTT TAATTATTAA ATCTGCTAAT GATATTGCTT CATATGGTTT TAAAGTTGCT      360
GGTGATCCTT CTATTAAGAA AGATGGATAT TTTAAAAAAT TGCAAGATGA ATTAGATAAT      420
ATTGTTGATA ATAATTCCGA TGATGATGCA ATAGCTAAG CTATTAAAGA TTTTAAAGCG      480
CGATGTGGTA TTTTAATTAA AGAAGCTAAA CAATATGAG AAGCTGCAAA AAATATTGTA      540
ACATCTTTAG ATCAATTTTT ACATGGTGAT CAGAAAAAAT TAGAAGGTGT TATCAATATT      600
CAAAAACGTT TAAAGAAGT TCAAACAGCT CTTAATCAAG CCCATGGGGA AAGTAGTCCA      660
GCTCATAAAG AGTTATTAGA AAAAGTAAAA AATTTAAAA CAACATTAGA AAGGACTATT      720
AAAGCTGAAC AAGATTTAGA GAAAAAAGTA GAATATAGTT TTCTATTAGG ACCATTGTTA      780
GGATTTGTTG TTTATGAAAT TCTTGAAAA ACTGCTGTTT AGCATATAAA AAATCAAATT      840
GATGAGATAA AGAAACAATT AGATTCTGCT CAGCATGATT TGGATAGAGA TGTTAAAAAT      900
ATAGGAATGT TAAATAGTAT TAATACAGAT ATTGATAATT TATATAGTCA AGGACAAGAA      960
GCAATTAAAG TTTTCCAAA GTTACAAGGT ATTTGGGCTA CTATTGGAGC TCAAATAGAA      1020
AATCTTAGAA CAACGTCGTT ACAAGAAGTT CAAGATTCTG ATGATGCTGA TGAGATACAA      1080
ATTGAACTTG AGGACGCTTC TGATGCTTGG TTAGTTGTGG CTCAAGAAGC TCGTGATTTT      1140
ACACTAAATG CTTATTCAAC TAATAGTAGA CAAAATTTAC CGATTAATGT TATATCAGAT      1200
TCATGTAATT GTTCAACAC AAATATGACA TCAAATCAAT ACAGTAATCC AACACAAAT      1260
ATGACATCAA ATCAATATAT GATTTCACAT GAATATACAA GTTTACCAA TAATTTTATG      1320
TTATCAAGAA ATAGTAATTT AGAATATAAA TGTCCTGAAA ATAATTTTAT GATATATTGG      1380
TATAATAATT CGGATTGGTA TAATAATTCG GATTGGTATA ATAAT      1425

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(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 475 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: BACILLUS THURINGIENSIS
- (C) INDIVIDUAL ISOLATE: PS86A1

(vii) IMMEDIATE SOURCE:

- (B) CLONE: E. coli NM522(pMYC1638) NRRL B-18751

(ix) FEATURE:

- (A) NAME/KEY: Protein
- (B) LOCATION: 1..475

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

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Met Ile Ile Asp Ser Lys Thr Thr Leu Pro Arg His Ser Leu Ile His
 1           5           10
Thr Ile Lys Leu Asn Ser Asn Lys Lys Tyr Gly Pro Gly Asp Met Thr
          20           25           30
Asn Gly Asn Gln Phe Ile Il Ser Lys ln Glu Trp Ala Thr Ile Gly
          35           40           45

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Ala Tyr Ile In Thr Gly Leu ly Leu Pro Val Asn Glu Gln Gln Leu
 50 55 60
 Arg Thr His Val Asn Leu Ser Gln Asp Ile Ser Ile Pro Ser Asp Phe
 65 70 75 80
 Ser Gln Leu Tyr Asp Val Tyr Cys Ser Asp Lys Thr Ser Ala Glu Trp
 85 90 95
 Trp Asn Lys Asn Leu Tyr Pro Leu Ile Ile Lys Ser Ala Asn Asp Ile
 100 105 110
 Ala Ser Tyr Gly Phe Lys Val Ala Gly Asp Pro Ser Ile Lys Lys Asp
 115 120 125
 Gly Tyr Phe Lys Lys Leu Gln Asp Glu Leu Asp Asn Ile Val Asp Asn
 130 135 140
 Asn Ser Asp Asp Asp Ala Ile Ala Lys Ala Ile Lys Asp Phe Lys Ala
 145 150 155 160
 Arg Cys Gly Ile Leu Ile Lys Glu Ala Lys Gln Tyr Glu Glu Ala Ala
 165 170 175
 Lys Asn Ile Val Thr Ser Leu Asp Gln Phe Leu His Gly Asp Gln Lys
 180 185 190
 Lys Leu Glu Gly Val Ile Asn Ile Gln Lys Arg Leu Lys Glu Val Gln
 195 200 205
 Thr Ala Leu Asn Gln Ala His Gly Glu Ser Ser Pro Ala His Lys Glu
 210 215 220
 Leu Leu Glu Lys Val Lys Asn Leu Lys Thr Thr Leu Glu Arg Thr Ile
 225 230 235 240
 Lys Ala Glu Gln Asp Leu Glu Lys Lys Val Glu Tyr Ser Phe Leu Leu
 245 250 255
 Gly Pro Leu Leu Gly Phe Val Val Tyr Gln Ile Leu Glu Asn Thr Ala
 260 265 270
 Val Gln His Ile Lys Asn Gln Ile Asp Glu Ile Lys Lys Gln Leu Asp
 275 280 285
 Ser Ala Gln His Asp Leu Asp Arg Asp Val Lys Ile Ile Gly Met Leu
 290 295 300
 Asn Ser Ile Asn Thr Asp Ile Asp Asn Leu Tyr Ser Gln Gly Gln Glu
 305 310 315 320
 Ala Ile Lys Val Phe Gln Lys Leu Gln Gly Ile Trp Ala Thr Ile Gly
 325 330 335
 Ala Gln Ile Glu Asn Leu Arg Thr Thr Ser Leu Gln Glu Val Gln Asp
 340 345 350
 Ser Asp Asp Ala Asp Glu Ile Gln Ile Glu Leu Glu Asp Ala Ser Asp
 355 360 365
 Ala Trp Leu Val Val Ala Gln Glu Ala Arg Asp Phe Thr Leu Asn Ala
 370 375 380
 Tyr Ser Thr Asn Ser Arg Gln Asn Leu Pro Ile Asn Val Ile Ser Asp
 385 390 395 400
 Ser Cys Asn Cys Ser Thr Thr Asn Met Thr Ser Asn Gln Tyr Ser Asn
 405 410 415
 Pro Thr Thr Asn Met Thr Ser Asn Gln Tyr Met Ile Ser His Glu Tyr
 420 425 430
 Thr Ser Leu Pro Asn Asn Phe Met Leu Ser Arg Asn Ser Asn Leu Glu
 435 440 445
 Tyr Lys Cys Pro Glu Asn Asn Phe Met Ile Tyr Trp Tyr Asn Asn Ser
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 Asp Trp Tyr Asn Asn Ser Asp Trp Tyr Asn Asn
 465 470 475

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 3471 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Bacillus thuringiensis*

(B) STRAIN: kumamotoensis

(C) INDIVIDUAL ISOLATE: PS50C

(vii) IMMEDIATE SOURCE:

(B) CLONE: E. coli NM522(pMYC2320) NRRL B-18769

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

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AATTATAAAG ATTATCTGAA AATGTCTGGG GGAGAGAATC CTGAATTATT TGGAAATCCG	180
GAGACGTTTA TTAGTTCATC CACGATTCAA ACTGGAATTG GCATTGTTGG TCGAATACTA	240
GGAGCTTTAG GGGTTCCATT TGCTAGTCAG ATAGCTAGTT TCTATAGTTT CATTGTTGGT	300
CAATTATGGC CGTCAAGGAG CGTAGATATA TGGGGAGAAA TTATGGAACG AGTGGAGAAA	360
CTCGTTGATC AAAAAATAGA AAAATATGTA AAAGATAAGG CTCTTGCTGA ATTAAAAGGG	420
CTAGGAAATG CTTTGGATGT ATATCAGCAG TCACTTGAAG ATTGGCTGGA AAATCGCAAT	480
GATGCAAGAA CTAGAAAGTG TGTTCCTAAT CAATTATAG CTTTAGATCT TAACTTTGTT	540
AGTTCBAATC CATCTTTTGC AGTATCCGGA CACGAAGTAC TATTATTAGC AGTATATGCA	600
CAGGCTGTGA ACCTACATTT ATTGTTATTA AGAGATGCTT CTATTTTGG AGAAGAGTGG	660
GGATTTACAC CAGGTGAAAT TTCTAGATTT TATAATCGTC AAGTGCAACT TACCGCTGAA	720
TATTCAGACT ATTGTGTAAA GTGGTATAAA ATCGGCTTAG ATAAATTGAA AGGTACCACT	780
TCTAAAAGTT GGCTGAATTA TCATCAGTTC CGTAGAGAGA TGACATTACT GGTATTAGAT	840
TTGGTGGCGT TATTTCCAAA CTATGACACA CATATGTATC CAATCGAAAC AACAGCTCAA	900
CTTACACGGG ATGTGTATAC AGATCCGATA GCATTTAACA TAGTGACAG TACTGGATTC	960
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CGTCGCCAC ACTTGTTTGA TATACTCAGC TCAGTAGAAA TTAATACAAG TAGAGGGGGT	1080
ATTACGTTAA ATAATGATGC ATATATAAAC TACTGGTCAG GACATACCTT AAAATATCGT	1140
AGAACAGCTG ATTCGACCGT AACATACACA GCTAATTACG GTCGAATCAC TTCAGAAAAG	1200
AATTCATTTG CACTTGAGGA TAGGGATATT TTTGAAATTA ATTCBACTGT GGCBAACCTA	1260
GCTAATTACT ACCAAAAGGC ATATGGTGTG CCGGGATCTT GOTTCCATAT GGTAAAAAGG	1320
GGAACCTCAT CAACAACAGC GTATTTATAT TCAAAAACAC ATACAGCTCT CCAAGGGTGT	1380
ACACAGGTTT ATGAATCAAG TGATGAAATA CCTCTAGATA GAACTGTACC GGTAGCTGAA	1440
AGCTATAGTC ATAGATTATC TCATATTACC TCCATTCTT TCTCTAAAA TGGGAGTGCA	1500
TACTATGGGA GTTCCCTGT ATTGTTTGG ACACATACTA GTGCGGATTT AAATAATACA	1560
ATATATTGAG ATAAAATCAC TCAAATTCCA GCGGTAAAGG GAGACATGTT ATATCTAGGG	1620
GGTCCGCTAG TACAGGGTCC TGGATTTACA GGAGGAGATA TATTAAAAAG AACCAATCCT	1680
AGCATATTAG GGAACCTTGC GGTACAGTA AATGGGTCGT TATCACAAG ATATCGTGTA	1740
AGAATTCGCT ATGCTCTAC AACAGATTTT GAATTTACTC TATACCTTGG CGACACAATA	1800
GAAAAAATA GATTTAACAA AACTATGGAT AATGGGGCAT CTTTAACGTA TGAAACATTT	1860
AAATTCGCAA GTTTCATTAC TGATTTCCAA TTCAGAGAAA CACAAGATAA AATACTCCTA	1920
TCCATGGGTG ATTTTAGCTC CGGTCAAGAA GTTTATATAG ACCGAATCGA ATTCATCCCA	1980
GTAGATGAGA CATATGAGGC GGAACAAGAT TTAGAAGCGG CGAAGAAAGC AGTGAATGCC	2040
TTGTTTACGA ATACAAAAGA TGGCTTACGA CCAGGTGTAA CGGATTATGA AGTAAATCAA	2100

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GCGGCAAACT TAGTGGAAATG CCTATCGGAT GATTATATATC CAAATGAAAA ACGATTGTTA 2160
TTTGATGCGG TGAGAGAGGC AAAACGCCTC AGTGGGGCAC GTAACCTACT ACAAGATCCA 2220
GATTTCCAAG AGATAAACGG AGAAAATGGA TGGGCGGCAA GTACGGGAAT TGAGATTGTA 2280
GAAGGGGATG CTGTATTAA AGGACGTTAT CTACGCCTAC CAGGTGCACG AGAAATTGAT 2340
ACGGAAACGT ATCCAACGTA TCTGTATCAA AAAGTAGAGG AAGGTGTATT AAAACCATAC 2400
ACAGATATA GACTGAGAGG GTTTGTGGGA AGTAGTCAAG GATTAGAAAT TTATACGATA 2460
CGTCACCAA CGAATCGAAT TGTAAAGAAT GTACCAGATG ATTTATTGCC AGATGTATCT 2520
CCTGTAAACT CTGATGGCAG TATCAATCGA TGCAGCGAAC AAAAGTATGT GAATAGCCGT 2580
TTAGAAGGAG AAAACCGTTC TGGTGATGCA CATGAGTTCT CGCTCCCTAT CGATATAGGA 2640
GAGCTGGATT ACAATGAAAA TGCAGGAATA TGGGTTGGAT TTAAGATTAC GGACCCAGAG 2700
GGATACGCAA CACTTGGAAA TCTTGAATTA GTCGAAGAGG GACCTTTGTC AGGAGACGCA 2760
TTAGAGCGCT TGCAAAGAGA AGAACAACAG TGGAAGATTC AAATGACAAG AAGACGTGAA 2820
GAGACAGATA GAAGATACAT GGCATCGAAA CAAGCGGTAG ATCGTTTATA TGCCGATTAT 2880
CAGGATCAAC AACTGAATCC TGATGTAGAG ATTACAGATC TTACTGCGGC TCAAGATCTG 2940
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ACGAAGTTTA CAGAATTAAC AGATCGACTC CAACAAGCGT GGAATTTGTA TGATCAGCGA 3060
AATGCCATAC CAAATGGTGA TTTTCGAAAT GGGTTAAGTA ATTGGAATGC AACGCCTGGC 3120
GTAGAAGTAC AACAAATCAA TCATACATCT GTCCTTGTA TTCCAAACTG GGATGAACAA 3180
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ATCACAAAAA CAGTGACATT CATCCGTAT ACAGATCAAA TGTGGATTGA AATAAGTGAA 3420
ACAGAAGGTA CGTTCTATAT AGAAAGTGA GAATTGATTG TAGACGTAGA C 3471

```

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1157 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (iii) HYPOTHETICAL: YES
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: *Bacillus thuringiensis*
 - (B) STRAIN: *kumamotoensis*
 - (C) INDIVIDUAL ISOLATE: PS50C
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: E. coli NM522(pMYC2320) NRRL B-18769
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

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Ser	Thr	Ser	Val	Ser	Ser	Asp	Ser	Asn	Arg	Tyr	Pro	Phe	Ala	Asn	Glu
			20					25				30			
Pro	Thr	Asp	Ala	Leu	Gln	Asn	Met	Asn	Tyr	Lys	Asp	Tyr	Leu	Lys	Met
		35					40					45			
Ser	Gly	Gly	Glu	Asn	Pro	Glu	Leu	Phe	Gly	Asn	Pro	Glu	Thr	Phe	Ile
	50					55				60					
Ser	Ser	Ser	Thr	Il	Gln	Thr	ly	Ile	Gly	Ile	Val	Gly	Arg	Ile	Leu
	65				70				75					80	
Gly	Ala	Leu	Gly	Val	Pro	Phe	Ala	Ser	Gln	Ile	Ala	Ser	Phe	Tyr	Ser
				85					90					95	

Phe Ile Val Gly Gln Leu Trp Pro Ser Lys Ser Val Asp Ile Trp Gly
 100 105 110
 Glu Ile Met Glu Arg Val Glu Lu Leu Val Asp Gln Lys Ile Lu Lys
 115 120 125
 Tyr Val Lys Asp Lys Ala Leu Ala Glu Leu Lys Gly Leu Gly Asn Ala
 130 135 140
 Leu Asp Val Tyr Gln Gln Ser Leu Glu Asp Trp Leu Glu Asn Arg Asn
 145 150 155 160
 Asp Ala Arg Thr Arg Ser Val Val Ser Asn Gln Phe Ile Ala Leu Asp
 165 170 175
 Leu Asn Phe Val Ser Ser Ile Pro Ser Phe Ala Val Ser Gly His Glu
 180 185 190
 Val Leu Leu Leu Ala Val Tyr Ala Gln Ala Val Asn Leu His Leu Leu
 195 200 205
 Leu Leu Arg Asp Ala Ser Ile Phe Gly Glu Glu Trp Gly Phe Thr Pro
 210 215 220
 Gly Glu Ile Ser Arg Phe Tyr Asn Arg Gln Val Gln Leu Thr Ala Glu
 225 230 235 240
 Tyr Ser Asp Tyr Cys Val Lys Trp Tyr Lys Ile Gly Leu Asp Lys Leu
 245 250 255
 Lys Gly Thr Thr Ser Lys Ser Trp Leu Asn Tyr His Gln Phe Arg Arg
 260 265 270
 Glu Met Thr Leu Leu Val Leu Asp Leu Val Ala Leu Phe Pro Asn Tyr
 275 280 285
 Asp Thr His Met Tyr Pro Ile Glu Thr Thr Ala Gln Leu Thr Arg Asp
 290 295 300
 Val Tyr Thr Asp Pro Ile Ala Phe Asn Ile Val Thr Ser Thr Gly Phe
 305 310 315 320
 Cys Asn Pro Trp Ser Thr His Ser Gly Ile Leu Phe Tyr Glu Val Glu
 325 330 335
 Asn Asn Val Ile Arg Pro Pro His Leu Phe Asp Ile Leu Ser Ser Val
 340 345 350
 Glu Ile Asn Thr Ser Arg Gly Gly Ile Thr Leu Asn Asn Asp Ala Tyr
 355 360 365
 Ile Asn Tyr Trp Ser Gly His Thr Leu Lys Tyr Arg Arg Thr Ala Asp
 370 375 380
 Ser Thr Val Thr Tyr Thr Ala Asn Tyr Gly Arg Ile Thr Ser Glu Lys
 385 390 395 400
 Asn Ser Phe Ala Leu Glu Asp Arg Asp Ile Phe Glu Ile Asn Ser Thr
 405 410 415
 Val Ala Asn Leu Ala Asn Tyr Tyr Gln Lys Ala Tyr Gly Val Pro Gly
 420 425 430
 Ser Trp Phe His Met Val Lys Arg Gly Thr Ser Ser Thr Thr Ala Tyr
 435 440 445
 Leu Tyr Ser Lys Thr His Thr Ala Leu Gln Gly Cys Thr Gln Val Tyr
 450 455 460
 Glu Ser Ser Asp Glu Ile Pro Leu Asp Arg Thr Val Pro Val Ala Glu
 465 470 475 480
 Ser Tyr Ser His Arg Leu Ser His Ile Thr Ser His Ser Phe Ser Lys
 485 490 495
 Asn Gly Ser Ala Tyr Tyr Gly Ser Phe Pro Val Phe Val Trp Thr His
 500 505 510
 Thr Ser Ala Asp Leu Asn Asn Thr Ile Tyr Ser Asp Lys Ile Thr Gln
 515 520 525
 Ile Pro Ala Val Lys Gly Asp Met Leu Tyr Leu Gly Gly Ser Val Val
 530 535 540
 Gln Gly Pro Gly Phe Thr Gly Gly Asp Ile Leu Lys Arg Thr Asn Pro
 545 550 555 560

Ser Ile Leu Gly Thr Ph Ala Val Thr Val Asn Gly Ser Leu Ser Gln
 565 570 575
 Arg Tyr Arg Val Arg Ile Arg Tyr Ala Ser Thr Thr Asp Phe Glu Phe
 580 585 590
 Thr Leu Tyr Leu Gly Asp Thr Ile Glu Lys Asn Arg Phe Asn Lys Thr
 595 600 605
 Met Asp Asn Gly Ala Ser Leu Thr Tyr Glu Thr Phe Lys Phe Ala Ser
 610 615 620
 Phe Ile Thr Asp Phe Gln Phe Arg Glu Thr Gln Asp Lys Ile Leu Leu
 625 630 635 640
 Ser Met Gly Asp Phe Ser Ser Gly Gln Glu Val Tyr Ile Asp Arg Ile
 645 650 655
 Glu Phe Ile Pro Val Asp Glu Thr Tyr Glu Ala Glu Gln Asp Leu Glu
 660 665 670
 Ala Ala Lys Lys Ala Val Asn Ala Leu Phe Thr Asn Thr Lys Asp Gly
 675 680 685
 Leu Arg Pro Gly Val Thr Asp Tyr Glu Val Asn Gln Ala Ala Asn Leu
 690 695 700
 Val Glu Cys Leu Ser Asp Asp Leu Tyr Pro Asn Glu Lys Arg Leu Leu
 705 710 715 720
 Phe Asp Ala Val Arg Glu Ala Lys Arg Leu Ser Gly Ala Arg Asn Leu
 725 730 735
 Leu Gln Asp Pro Asp Phe Gln Glu Ile Asn Gly Glu Asn Gly Trp Ala
 740 745 750
 Ala Ser Thr Gly Ile Glu Ile Val Glu Gly Asp Ala Val Phe Lys Gly
 755 760 765
 Arg Tyr Leu Arg Leu Pro Gly Ala Arg Glu Ile Asp Thr Glu Thr Tyr
 770 775 780
 Pro Thr Tyr Leu Tyr Gln Lys Val Glu Glu Gly Val Leu Lys Pro Tyr
 785 790 795 800
 Thr Arg Tyr Arg Leu Arg Gly Phe Val Gly Ser Ser Gln Gly Leu Glu
 805 810 815
 Ile Tyr Thr Ile Arg His Gln Thr Asn Arg Ile Val Lys Asn Val Pro
 820 825 830
 Asp Asp Leu Leu Pro Asp Val Ser Pro Val Asn Ser Asp Gly Ser Ile
 835 840 845
 Asn Arg Cys Ser Glu Gln Lys Tyr Val Asn Ser Arg Leu Glu Gly Glu
 850 855 860
 Asn Arg Ser Gly Asp Ala His Glu Phe Ser Leu Pro Ile Asp Ile Gly
 865 870 875 880
 Gln Leu Asp Tyr Asn Glu Asn Ala Gly Ile Trp Val Gly Phe Lys Ile
 885 890 895
 Thr Asp Pro Glu Gly Tyr Ala Thr Leu Gly Asn Leu Glu Leu Val Glu
 900 905 910
 Glu Gly Pro Leu Ser Gly Asp Ala Leu Glu Arg Leu Gln Arg Glu Glu
 915 920 925
 Gln Gln Trp Lys Ile Gln Met Thr Arg Arg Arg Glu Glu Thr Asp Arg
 930 935 940
 Arg Tyr Met Ala Ser Lys Gln Ala Val Asp Arg Leu Tyr Ala Asp Tyr
 945 950 955 960
 Gln Asp Gln Gln Leu Asn Pro Asp Val Glu Ile Thr Asp Leu Thr Ala
 965 970 975
 Ala Gln Asp Leu Ile Gln Ser Ile Pro Tyr Val Tyr Asn Glu Met Phe
 980 985 990
 Pro lu Ile Pro Gly Met Asn Tyr Thr Lys Phe Thr Glu Leu Thr Asp
 995 1000 1005
 Arg Leu Gln ln Ala Trp Asn Leu Tyr Asp ln Arg Asn Ala Ile Pro
 1010 1015 1020

Asn Gly Asp Phe Arg Asn Gly Leu Ser Asn Trp Asn Ala Thr Pro Gly
 1025 1030 1035 1040
 Val lu Val Gln Gln Ile Asn His Thr Ser Val Leu Val Ile Pro Asn
 1045 1050 1055
 Trp Asp Glu Gln Val Ser Gln Gln Phe Thr Val Gln Pro Asn Gln Arg
 1060 1065 1070
 Tyr Val Leu Arg Val Thr Ala Arg Lys Glu Gly Val Gly Asn Gly Tyr
 1075 1080 1085
 Val Ser Ile Arg Asp Gly Gly Asn Gln Ser Glu Thr Leu Thr Phe Ser
 1090 1095 1100
 Ala Ser Asp Tyr Asp Thr Asn Gly Val Tyr Asn Asp Gln Thr Gly Tyr
 1105 1110 1115 1120
 Ile Thr Lys Thr Val Thr Phe Ile Pro Tyr Thr Asp Gln Met Trp Ile
 1125 1130 1135
 Glu Ile Ser Glu Thr Glu Gly Thr Phe Tyr Ile Glu Ser Val Glu Leu
 1140 1145 1150
 Ile Val Asp Val Glu
 1155

Claims

- 1 1. A method for controlling acarid pests wherein said method comprises contacting
2 said pests with an acarid-controlling effective amount of a B.t. endotoxin.
- 1 2. The method, according to claim 1, wherein said toxin is obtainable from a B.
2 t. isolate selected from the group consisting of B.t. PS50C, B.t. PS86A1, B.t. PS69D1, B.t.
3 PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t. PS24J, B.t. PS94R3, B.t. PS17, B.t. PS62B1
4 and B.t. PS74G1, and mutants thereof.
- 1 3. The method, according to claim 2, wherein said isolate is PS50C.
- 1 4. The method, according to claim 2, wherein said isolate is PS86A1.
- 1 5. The method, according to claim 2, wherein said isolate is PS69D1.
- 1 6. The method, according to claim 2, wherein said isolate is PS72L2.
- 1 7. The method, according to claim 2, wherein said isolate is PS75J2.
- 1 8. The method, according to claim 2, wherein said isolate is PS83E5.
- 1 9. The method, according to claim 2, wherein said microbe is PS45B1.
- 1 10. The method, according to claim 2, wherein said isolate is PS24J.
- 1 11. The method, according to claim 2, wherein said isolate is PS94R3.
- 1 12. The method, according to claim 2, wherein said isolate is PS17.
- 1 13. The method, according to claim 2, wherein said isolate is PS62B1.
- 1 14. The method, according to claim 2, wherein said isolate is PS74G1.
- 1 15. The method, according to claim 3, wherein said toxin has the amino acid
2 sequence of SEQ ID NO. 28.

1 16. The method, according to claim 4, wherein said toxin has the amino acid
2 sequence of SEQ ID NO. 30.

1 17. The method, according to claim 5, wherein said toxin has the amino acid
2 sequence of SEQ ID NO. 10.

1 18. The method, according to claim 12, wherein said toxin has the amino acid
2 sequence of SEQ ID NO. 2.

1 19. The method, according to claim 12, wherein said toxin has the amino acid
2 sequence of SEQ ID NO. 4.

1 20. The method, according to claim 1, wherein said acarid pest is a mite.

1 21. The method, according to claim 20, wherein said mite is the Two Spotted
2 Spider Mite.

1 22. A composition of matter comprising a Bacillus thuringiensis isolate selected
2 from the group consisting of B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t. PS24J,
3 B.t. PS94R3, B.t. PS62B1 and B.t. PS74G1, and mutants thereof, or proteins, toxic crystals, or
4 spores of said isolates, in association with an inert carrier.

1 23. The composition of matter, according to claim 22, comprising Bacillus
2 thuringiensis PS24J.

1 24. The composition of matter, according to claim 22, comprising Bacillus
2 thuringiensis PS94R3.

 25. The composition of matter, according to claim 22, comprising Bacillus
thuringiensis PS45B1.

1 26. The composition of matter, according to claim 22, comprising Bacillus
2 thuringiensis PS62B1.

1 27. The composition of matter, according to claim 22, comprising Bacillus
2 thuringiensis PS74G1.

1 28. The composition of matter, according to claim 22, comprising Bacillus
2 thuringiensis PS72L1.

1 29. The composition of matter, according to claim 22, comprising Bacillus
2 thuringiensis PS75J1.

1 30. The composition of matter, according to claim 22, comprising Bacillus
2 thuringiensis PS83E5.

1 31. A composition for controlling an acaride pest wherein said composition
2 comprises substantially intact, treated cells having pesticidal activity and prolonged persistence
3 in the feeding zone of said pests when applied to the environment of acaride pests, wherein
4 said pesticide is a polypeptide toxic to acaride pests, is intracellular, and is produced by a
5 Bacillus thuringiensis isolate selected from the group consisting of B.t. PS50C, B.t. PS86A1,
6 B.t. PS69D1, B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t. PS24J, B.t. PS94R3, B.t.
7 PS17, B.t. PS62B1 and B.t. PS74G1, and mutants thereof.

1 32. The pesticidal composition, according to claim 18, wherein said treated cells
2 are treated by chemical or physical means to prolong the pesticidal activity in the environment.

1 33. A gene encoding a toxin which is active against acarides wherein said gene is
2 obtainable from a Bacillus thuringiensis isolate selected from the group consisting of B.t.
3 PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t. PS24J, B.t. PS94R3, B.t. PS62B1 and B.t.
4 PS74G1, and mutants thereof or is equivalent to one of said genes.

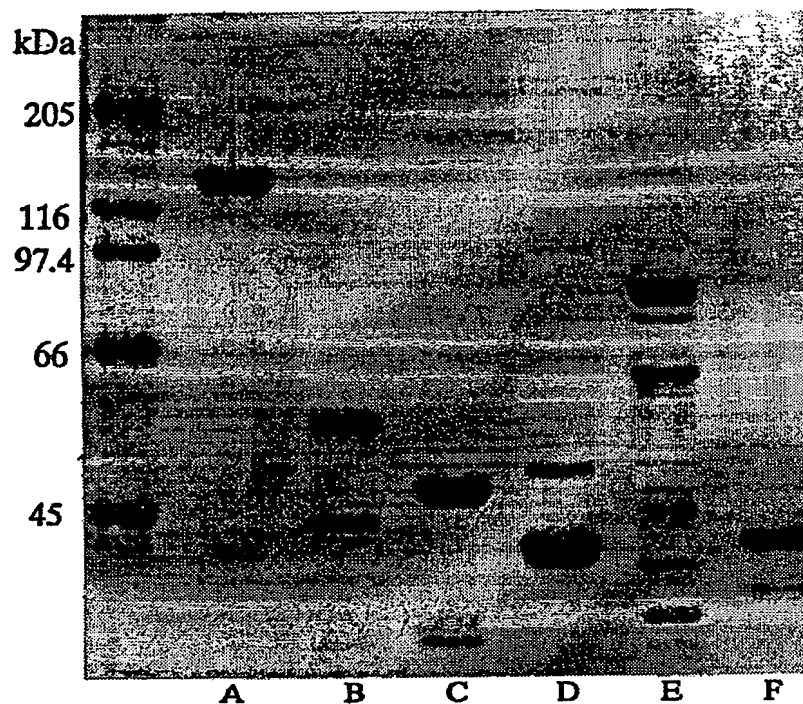
1 34. A toxin encoded by a gene obtainable from a Bacillus thuringiensis isolate
2 selected from the group consisting of B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t.
3 PS24J, B.t. PS94R3, B.t. PS62B1 and B.t. PS74G1, and mutants thereof, wherein said toxin is
4 active against acaride pests.

1 35. A transformed host selected from the group consisting of a plant, a microbe,
2 and a baculovirus transformed by a gene obtainable from a Bacillus thuringiensis isolate
3 selected from the group consisting of B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t.
4 PS24J, B.t. PS94R3, B.t. PS62B1 and B.t. PS74G1, and mutants thereof.

- 1 36. A biologically pure culture of a Bacillus thuringiensis selected from the group
- 2 consisting of B.t. PS72L1, B.t. PS75J1, B.t. PS83E5, B.t. PS45B1, B.t. PS24J, B.t. PS94R3, B.t.
- 3 PS62B1 and B.t. PS74G1, and mutants thereof.

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Figure 1

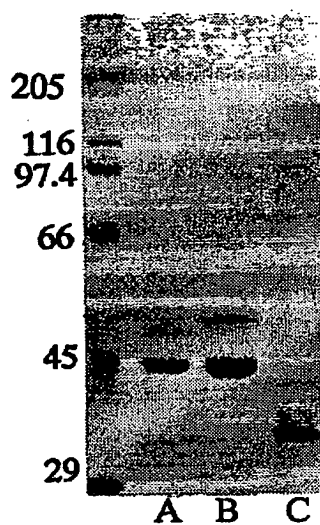


- A. *Bacillus thuringiensis* PS50C
- B. *Bacillus thuringiensis* PS86A1
- C. *Bacillus thuringiensis* PS69D1
- D. *Bacillus thuringiensis* PS72L1
- E. *Bacillus thuringiensis* PS75J1
- F. *Bacillus thuringiensis* PS83E5

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Figure 2A

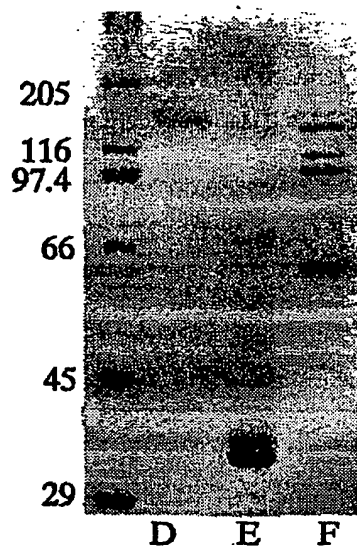


- A. *Bacillus thuringiensis* PS24J
- B. *Bacillus thuringiensis* PS94K3
- C. *Bacillus thuringiensis* PS45B1

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Figure 2B



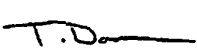
- D. *Bacillus thuringiensis* PS17
- E. *Bacillus thuringiensis* PS62B1
- F. *Bacillus thuringiensis* PS74C1

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INTERNATIONAL SEARCH REPORT

PCT/US 92/03546

International Application No.

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all) ⁶		
According to International Patent Classification (IPC) or to both National Classification and IPC		
Int.Cl. 5 A01N63/00; C12R1:07)	A01N63/02;	C12N1/20; //(C12N1/20,
II. FIELDS SEARCHED		
Minimum Documentation Searched ⁷		
Classification System	Classification Symbols	
Int.Cl. 5	A01N ; C12R	
Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ⁸		
III. DOCUMENTS CONSIDERED TO BE RELEVANT⁹		
Category ¹⁰	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
X	US,A,4 849 217 (G.G.SOARES ET. AL.) 18 July 1989 cited in the application see column 2, line 28 - column 3, line 40 see column 4, line 6 - column 5, line 25; claims ---	22, 25-27, 31,32,36
A	EP,A,0 303 426 (MYCOGEN CORPORATION) 15 February 1989 ---	
A	JOURNAL OF ECONOMIC ENTOMOLOGY. vol. 83, no. 3, 1990, COLLEGE PARK, MARYLAND US pages 792 - 798; R.N.ROYALTY ET. AL.: 'Effects of Thuringiensin on Tetranychus urticae (Acari: Tetranychidae) Mortality, Fecundity, and Feeding.' cited in the application --- -/-	
<p>¹⁰ Special categories of cited documents:</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"A" document member of the same patent family</p>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report	
16 SEPTEMBER 1992	25.09.92	
International Searching Authority	Signature of Authorized Officer	
EUROPEAN PATENT OFFICE	DONOVAN T.M. 	

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		- Relevant to Claim No.
Category *	Citation of Document, with indication, where appropriate, of the relevant passages	
A	<p>CHEMICAL ABSTRACTS, vol. 101, no. 23, 3 December 1984, Columbus, Ohio, US; abstract no. 206066P, R.A.LI ET. AL.: 'Biology of ectoparasites of birds and acaricides to control them.' page 222 ; see abstract & VET. ENTOMOL. AKAROL. 1983, USSR pages 263 - 272;</p> <p>---</p>	
A	<p>JOURNAL OF INVERTEBRATE PATHOLOGY vol. 56, no. 2, September 1990, pages 258 - 266; S.C.MACINTOSH ET. AL.: 'Specificity and Efficacy of Purified Bacillus thuringiensis Proteins against Agronomically Important Insects'</p> <p>---</p>	

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ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO.

US 9203546
SA 60534

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 16/09/92

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EP-A-0303426	15-02-89	US-A- 4948734	14-08-90
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